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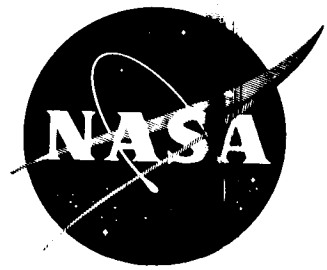

PROCEEDINGS OF THE MICROBIAL RESPONSE TO SPACE ENVIRONMENT SYMPOSIUM

Conducted at the
NASA Lyndon B. Johnson Space Center
Houston, Texas, September 1972

Edited by
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16. Abstract

An experiment designed to evaluate the response of nine different species to the space flight environment was conducted as part of the Apollo 16 mission. Evaluations were based on model systems correlated with disease or other medically important conditions that could affect the health of future space travelers. Two months after the recovery of the Apollo 16 spacecraft, a symposium was conducted at the NASA Lyndon B. Johnson Space Center to discuss early findings of the experiment. The data presented at this symposium, which represents a minor portion of the complete study, are detailed in this report. Each section has been prepared by the scientist who is most responsible for the performance of a specific task.

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PROCEEDINGS OF THE MICROBIAL RESPONSE TO SPACE ENVIRONMENT SYMPOSIUM

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CONTENTS

SECTION I EXPERIMENT DESIGN AND HARDWARE

BACKGROUND AND GENERAL DESIGN OF THE MICROBIAL RESPONSE TO SPACE ENVIRONMENT EXPERIMENT (M191) SYSTEM

By Gerald R. Taylor
Lyndon B. Johnson Space Center

ABSTRACT

Nine different species of organisms were exposed to space flight conditions during the Apollo 16 mission. Each test system was selected because it provided a quantitative method of evaluating some medically important phenomenon. The experiment design and each of the test systems are discussed.

SUMMARY

Early U.S. and U.S.S.R. studies have established the ability of microbes to survive the space environment although actual survival rates were often different from ground controls. More recent space flight studies have produced data that suggest the possibility of space flight mediated genetic and developmental alterations in several species. The Microbial Response to Space Environment Experiment was designed to evaluate the relationship between space flight and viability and to study the effect of space flight on phenotypic expressions in several biological systems.

BACKGROUND AND INTRODUCTION

Many ground-based microbiological studies have been conducted to evaluate the effects of simulated space flight environments on medically important microorganisms. A common practice has been to simulate the confinement and reduced atmospheric pressures of space vehicles by placing the test systems in small decompression chambers. By using this method in several studies, a positive relationship between space flight conditions and the rate of infection from microbial sources has been established in recent years. Berry (ref. 1) observed an increase in infection by Salmonella typhimurium in mice exposed to a simulated altitude of

6096 meters (20 000 feet). Giron et al. (ref. 2) found increased morbidity in mice after intraperitoneal injection of mengovirus at a simulated altitude of 5486.4 meters (18 000 feet). In studies of mice injected subcutaneously with Staphylococcus aureus at a simulated altitude of 8229.6 meters (27 000 feet), Schmidt et al. (ref. 3) demonstrated that lesions developing on test animals were larger and healed at a slower rate than on control animals. The work of Ehrlich and Mieszkuc (refs. 4 and 5) demonstrated that simulated space cabin environments at 5486.4-meter (18 000 foot) and 10 668-meter (35 000 foot) altitude equivalents increased the mortality of mice infected with Klebsiella pneumoniae. The 10 668-meter (35 000 foot) altitude environment also reduced the effectiveness of lincomycin as a treatment for Staphylococcus aureus infections (ref. 6). These authors also reported increased mortality of mice because of influenza virus infection when the mice were maintained in a simulated spacecraft environment of 34 475 N/m^2 (5 psi) and 100 percent oxygen (ref. 7). These and many other studies have repeatedly signaled the importance of conducting meaningful microbiological investigations in conjunction with manned and unmanned space flights.

In accordance with this recognized importance of conducting space microbiology studies, numerous different microbial species have been flown on many Soviet and American space flights (tables I to V). Most of these microbiology studies were concerned with establishing the now-accepted principle that microbes can survive in the harsh environment of space. However, certain anomalies noticed in these studies suggested that the survival of some microbes was affected synergistically, whereas other microbes were affected adversely by the space environment. For example, Glembotskiy et al. (ref. 8) report quite different results after exposure of aqueous suspensions of spores from members of the genus Streptomyces (Actinomyces in the U.S.S.R.) to space flight conditions on board the third, fourth, and fifth Soviet satellites. With S. erythraeus strain 2577, the authors reported that space flight conditions increased the incidence of spore germination by approximately six times that of the ground-based controls, whereas spore germination decreased significantly with S. erythraeus strain 8594 and S. aureofaciens LSB 2201. These authors reported that space flight conditions stimulated mycelial growth of S. aureofaciens. In another example, Kovyazin et al. (ref. 9) have reported that the viability of diploid cells of Saccharomyces vini megri strain 139-13 sensitized with olic acid was unaffected by the conditions of space flight, whereas similarly sensitized haploid cells of S. cerevisiae strain 40-2587 sustained considerable loss of viability.

These examples are typical of past survival studies, in which results were quite evenly divided among synergism, antagonism, or no relationship at all between space flight and microbial viability (refs. 8 to 14). Unfortunately, most of these studies were hindered by technical constraints, mission anomalies, or inconsistent controls. Thus, equivocal results were often produced despite the best effort of the investigators.

A few of the more recent American and Soviet microbiology studies have been used to investigate the effects of space flight on parameters other than viability. Generally, these studies have involved genetic changes and, as with the survival studies, they have produced variable results (refs. 10, 12, 13, and 15 to 20). For example, phage induction in Escherichia coli and Salmonella typhimurium as related to strontium-85 (^{85}Sr) gamma irradiation was studied by Mattoni (ref. 12) in the American Biosatellite II P-1135 experiment. It was reported that: "Under space flight conditions, induction was increasingly less frequent with increasing radiation when compared to ground controls" (ref. 12). In contrast to this finding, Antipov (ref. 16) and Zhukov-Verezhnikov, et al. (ref. 21) reported studies involving the induction of Escherichia coli bacteriophage K-12 (λ) on board the Vostok 1, 2, 3, 4, 5, and 6 spacecraft; phage induction increased as a function of mission duration (fig. 1).

Although the studies just mentioned sometimes appear to be contradictory, the combined results of these studies are overwhelmingly suggestive of synergistic or antagonistic relationships between microbial genetic alterations and space flight conditions. This situation was recognized by the National Academy of Sciences (NAS), which observed that: "The possibility that the special conditions of long-duration space missions may give rise to microbial mutants must be carefully considered" (ref. 22). The NAS recommended that future experimentation should "investigate the effect of spacecraft conditions on the rate of mutations in different microorganisms ..." (ref. 22). The Microbial Response to Space Environment Experiment (M191) was conducted in accordance with this recommendation.

GENERAL EXPERIMENT DESIGN

From the multitude of microbial species and challenge systems that are available, the experiment system outlined in table VI was established. In most cases, the phenomena studied represent well-known model systems that can be correlated directly with disease or other medically important conditions that could affect the health of future astronauts. Experts from the scientific community were invited to study those phenomena within their area of expertise and to conduct critical investigations in their own laboratories. This method facilitated the performance of a large number of individual studies in a coordinated manner and permitted a variety of species to be housed within a single piece of flight hardware. Each investigator selected a species of microorganism that was nonpathogenic to man (to avoid possible contamination of the crewmen), that was well characterized relative to the phenomenon to be studied, that was well suited to simple and rapid screening tests, and that was compatible with the unique environment of the flight hardware.

Aeromonas proteolytica was chosen because it produces two exotoxins of major medical importance. One exotoxin is an endopeptidase that quantitatively degrades hemoglobin and causes intracutaneous hemorrhage and necrosis in laboratory animals (ref. 23). The other exotoxin, introduced into the culture fluid, has the ability to hemolyse human erythrocytes (ref. 23). Cells recovered from the flight hardware were evaluated critically for alterations in toxin production.

Of several fungi that were considered, four different species were selected. Trichophyton terrestre was included because, under laboratory conditions, it attacks human hair. The other filamentous fungus, Chaetomium globosum, was of special interest because of its cellulolytic activity on cloth fibers similar to those that are contained in portions of the crewmember flight garments. The two yeasts, Rhodotorula rubra and Saccharomyces cerevisiae, were well suited to drug sensitivity studies.

Nematospiroides dubius was the only multicellular organism used in this study. This nematode has been cultured successfully in vitro from the egg to the third stage infective larvae (ref. 24). Nematospiroides dubius is pathogenic to laboratory mice but not to humans and is insensitive to the special holding conditions of the flight hardware. Specimens subjected to the space flight experiment conditions were evaluated for changes in survival, infectivity in mice, formation of adults, egg production, and egg development.

Bacillus subtilis was chosen for study by two different investigative groups because of the known stability of this species in extreme environments and because of the copious investigations that have been conducted regarding this microorganism. One group evaluated the survival of this microorganism under space flight conditions and correlated these findings with those obtained for the same strain employed in the Apollo 16 biostack flight experiment. The other group chose strains HA 101 (59) and HA 101 (59)F because both strains have three specific amino acid markers and because the HA 101 (59)F strain is defective in the ability to repair radiation damage and is, therefore, highly susceptible to the damaging effects of ultraviolet (uv) irradiation (ref. 25). Returned cells were subjected to extensive somatic and genetic evaluations.

Bacillus thuringiensis var. thuringiensis was selected because it produces a biologically active β-exotoxin, a lipolytic α-exotoxin, and a crystalline δ-endotoxin, and because the organism has been used widely as a biological insecticide (ref. 26). Several in vitro and in vivo systems, including reactions in the silkworm and housefly, were used to evaluate possible alterations in toxin production.

Survival studies of the T-7 bacteriophage of Escherichia coli were included to relate the present experiment to the space-flight-mediated

effects reported by the Soviets for the Escherichia coli phage that were flown on numerous manned flights (refs. 11, 16, 21, and 27). Rather than the T-1 or K-12 (λ) phage commonly employed by the Soviets, the simpler and more stable T-7 phage was chosen for this study in the hope that it would be more resistant to the rigors of space flight and, therefore, prove to be a better uv test subject.

To allow for dose-response studies and comparative investigations, certain variables were provided within the flight hardware. Microbes could be suspended in 0.05 cubic centimeter (50 microliters) of fluid or could be dried on a suitable carrier. Some of the microbes were exposed to the vacuum of space, whereas other microbes were retained at a pressure of 1 atmosphere. Because detailed genetic studies required exposure to a mutagenic source, provisions were made to expose test systems to the full light of space or to components of the solar uv spectrum at peak wavelengths of 254, 280, and 300 nanometers. An optical filtering system was provided to control the total energy reaching exposed test systems from a minimum of 4×10^{-6} joule/cm^2 (4×10^1 ergs/cm^2) to a maximum of 8×10^1 joules/cm^2 (8×10^8 ergs/cm^2). The use of ambient solar energy as the mutagen necessitated close monitoring. Photographic emulsion and a modification of the potassium ferrioxalate system of Wrighton and Witz (ref. 28) were used to record the amount of energy that actually reached selected test systems (table VII).

The possible mutagenic activity of galactic irradiation necessitated the inclusion of lithium fluoride thermoluminescent dosimeters and a package of passive nuclear-track detectors capable of recording high-energy multicharged particles (table VII). These studies were conducted in a manner that allowed for direct correlation with similar readings recovered from the biostack, the Apollo crewmen personal radiation dosimeters, and the Apollo Light Flash Moving Emulsion Detector (ALFMED), all of which were used in the Apollo 16 command module.

The fact that each of these varied and complex test systems functioned optimally without any serious loss of data is a tribute to the dedication and support given by every investigator associated with this experiment. There can be no question that the experiment objectives were accomplished in a singular and unequivocal manner.

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TABLE I.- SPACE-FLOWN MICROORGANISMS (VIRUSES)

Microorganism	Flight	Condition	Reference
Tobacco mosaic virus Influenza virus	U.S.S.R.	(a)	10 and 15
Poliomyelitis virus	U.S. Balloon	34 to 155 km, altitude	10
Tobacco mosaic virus	Gemini IXA	Dry	11
	Gemini X/ Agena VIII		29
	Gemini XII		27
Vaccinia virus	Gemini XII	Dry	27
Influenza (PR-8 strain)	Gemini XII	Dry	29
Canine hepatitis			27
Infectious bovine Rhinotracheitis			30

[a]Unknown.

TABLE II.- SPACE-FLOWN MICROORGANISMS (BACTERIA/PHAGE)

Microorganism	Flight	Condition	Reference
Escherichia coli K-12/K-12	Sputnik 4 and 5	(a)	16, 17, 15
	Vostok 1, 2, 3, 4, 5, 6	Nutrient suspension ^{60}Co-γ	
	Cosmos 110	Nutrient suspension ^{60}Co-γ	
Escherichia coli T_4	U.S. balloon	34 to 155 km, altitude	10
Escherichia coli C-600	Biosatellite II P-1135	Growing in liquid ^{85}Sr-γ	12
Salmonella typhimurium BS-5(P-22)/P-22	Biosatellite II P-1135	Growing in liquid ^{85}Sr-γ	13
Escherichia coli T_1	U.S. balloon Aerobee Gemini IXA Gemini X/ Agena VIII Gemini XII	Dry	29, 27
	Sputnik 5 and 6 Voskhod 1 and 2	Dry	15
Aerobacter aerogenes 1321	Vostok 2	(a)	10
Escherichia coli B/T_2	Vostok 2	(a)	31

[a]Unknown.

TABLE III.- SPACE-FLOWN MICROORGANISMS (BACTERIA)

Microorganism	Flight	Condition	Reference
Escherichia coli K-12 (λ) Aerobacter aerogenes 1321 Escherichia coli B Staphylococcus aureus 0.15	Vostok 1	Agar cultures	10 and 31
Clostridium butyricum	Vostok 1	Spore suspension	10
Bacillus brevis	Voskhod 1	Spores	
Clostridium sporogenes	Discoverer XVII Discoverer XVIII	(a)	
Bacillus subtilis ATCC 6052	Gemini IXA Gemini X/ Agena VIII Gemini XII	Dry	11 29 27

[a]Unknown.

TABLE IV.- SPACE-FLOWN MICROORGANISMS

(*Streptomyces* (*Actinomyces*))

Microorganism	Flight	Condition	Reference
S. erythraeus 2577 *S. erythraeus* 8594	Vostok 2	Aqueous spore suspension and liquid mycelium suspension	8
S. streptomycini kras LS-3	Vostok 2	(a)	14
S. aureofaciens LSB 2201	Vostok 4 Vostok 5	Aqueous spore	8

[a]Unknown.

TABLE V.- SPACE-FLOWN MICROORGANISMS (YEASTS AND FUNGI)

Microorganism	Flight	Condition	Reference
Saccharomyces cerevisiae 40-2587 (haploid) Saccharomyces vini megri 139-13 strain (diploid)	Vostok 2 Voskhod 1	Suspensions both unsensitized and sensitized with olic acid	9 and 10
Penicillium roqueforti	U.S. balloon	Dry spores (34 km altitude for 6 hr)	10 and 27
	Gemini XII	Dry spores	27
Neurospora crassa	Biosatellite II P-1037	Dry spores ^{85}Sr-γ	19
Penicillium roqueforti thom	Gemini IXA Gemini X/ Agena VIII	Dry spores	29 and 27
Neurospora sp.	U.S. balloon	(a)	27
	Gemini XI	Dry spores phosphorus-32 (^{32}P)-γ and metabolizing spore suspension ^{32}P-γ	13
	Nerv I	1900 km altitude for 28 min	15
	Discoverer XVIII	Dry spores	

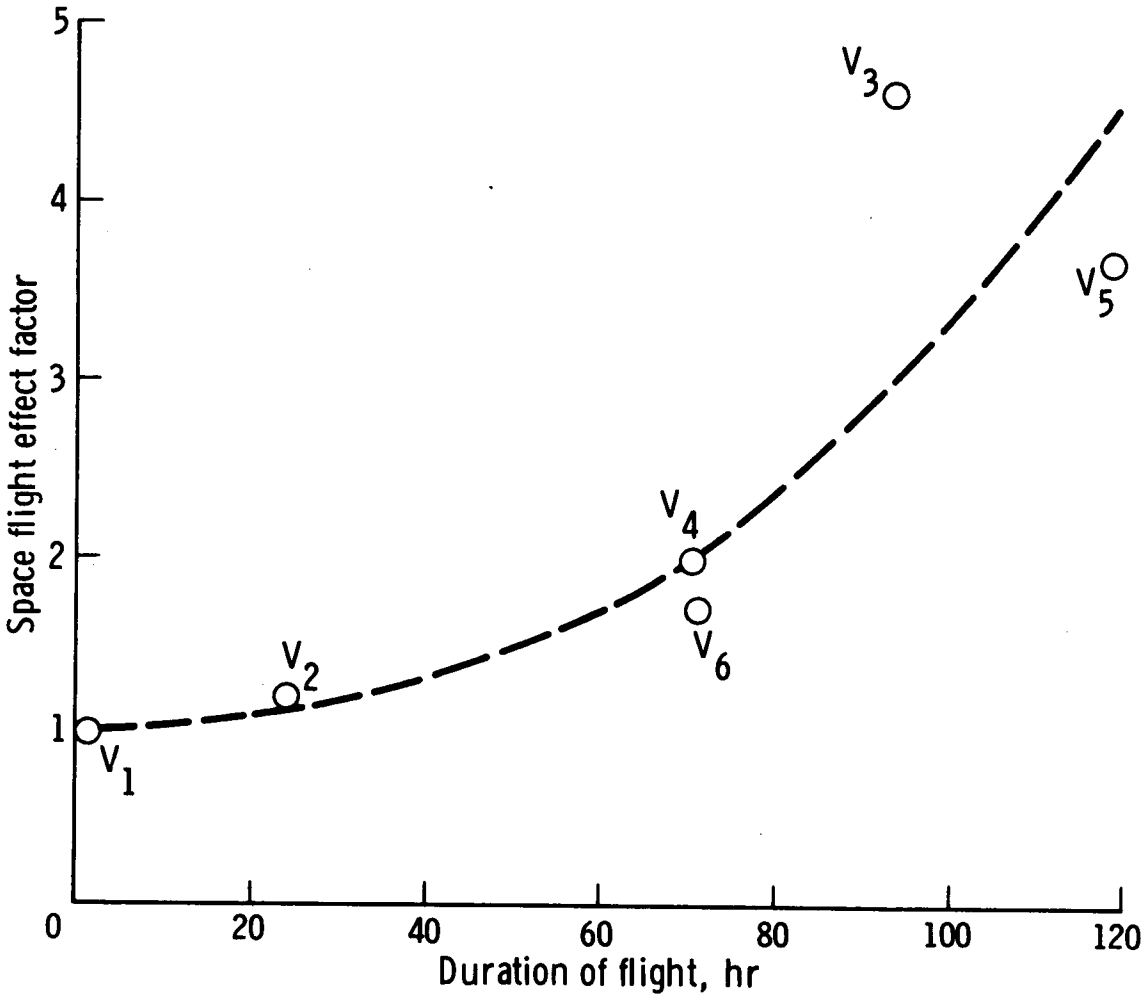
[a]Unknown.

TABLE VI.- BIOLOGICAL COMPONENTS AND INVESTIGATORS FOR EXPERIMENT M191

Phenomenon studied	Assay system	Microorganism	Investigator
Hemorrhagic factor production Hemolytic enzyme production	Guinea pig and hemoglobin Human erythrocytes	Aeromonas proteolytica	B. G. Foster, D. O. Lovett Texas A. & M. University College Station, Texas
Cellulolytic activity	Cloth fibers	Chaetomium globosum	P. A. Volz, Y. C. Hsu, D. E. Jerger, J. L. Hiser, J. M. Veselenak Eastern Michigan University Ypsilanti, Michigan
Animal tissue invasion	Human hair	Trichophyton terrestre	
Drug sensitivity	Antibiotic sensitivity in agar	Rhodotorula rubra Saccharomyces cerivisiae	
Infectivity	Mouse	Nematospiroides dubius	R. A. Long, W. L. Ellis Northrop Services, Inc. Houston, Texas G. R. Taylor NASA Lyndon B. Johnson Space Center Houston, Texas
Genome alteration	Spore production	Bacillus subtilis spores, strains HA 101 (59) and HA 101 (59)F	J. Spizizen, J. E. Isherwood Scripps Clinic and Research Foundation La Jolla, California
uv and vacuum sensitivity	Colony formation	Bacillus subtilis spores, strain 168	H. Bücker, G. Horneck, H. Wollenhaupt University of Frankfurt, Germany
Lipolytic α-toxin production Deforming β-toxin production Fatal δ-toxin production	Lytic zone on agar Sarcina flava and housefly Silkworm and crystal assay	Bacillus thuringiensis	R. T. Wrenn, W. L. Ellis Northrop Services, Inc. Houston, Texas G. R. Taylor, R. C. Simmonds NASA Lyndon B. Johnson Space Center Houston, Texas A. M. Heimpel U.S. Dept. of Agriculture Beltsville, Maryland
Bacteria phage infectivity	Host lysis	Escherichia coli (T-7 phage)	J. Spizizen, J. E. Isherwood Scripps Clinic and Research Foundation La Jolla, California

TABLE VII.- EXPERIMENT M191 DOSIMETRY COMPONENTS

Measurement	Monitor used	Assay system	Investigator
High-energy multi-charged particles	Passive nuclear-track detectors	Lexan Cellulose nitrate Photographic emulsion Silver chloride	E. V. Benton University of San Francisco San Francisco, California
Ultraviolet light	Passive dosimeters	Potassium ferrioxalate actinometry Photographic emulsion	M. B. Parson, R. A. Long, W. Ellis Northrop Services, Inc. Houston, Texas G. R. Taylor NASA Lyndon B. Johnson Space Center Houston, Texas
Penetration of galactic irradiation	Thermoluminescent dosimeters	Lithium fluoride	J. V. Bailey NASA Lyndon B. Johnson Space Center Houston, Texas R. A. English, R. D. Brown Kelsey-Seybold Clinic Houston, Texas



Notes:
1. Data from reference 15.
2. V_1 to V_6 denote Vostok flight number.
3. Space flight effect factor = number of phage particles/ground control cell.

Figure 1.- Effect of duration of Vostok space flight missions on K-12 (λ) phage induction in Escherichia coli.

DESCRIPTION OF THE MICROBIAL ECOLOGY EVALUATION DEVICE, FLIGHT EQUIPMENT, AND GROUND TRANSPORTER

By Charles E. Chassay and Gerald R. Taylor
Lyndon B. Johnson Space Center

ABSTRACT

Exposure of test systems in space required the fabrication of specialized hardware termed a Microbial Ecology Evaluation Device that had individual test chambers and a complex optical filter system. The characteristics of this device and the manner in which it was deployed in space are described.

SUMMARY

During the extravehicular activity conducted as part of the Apollo 16 mission, a Microbial Ecology Evaluation Device, containing some 20 million microbes, was attached to the opened command module hatch door and exposed to the environment of space. This device was specially designed to expose various test systems to different components of the solar ultraviolet spectrum and to the vacuum of space. The varied requirements of the investigators necessitated design and fabrication of a rather complex device that was capable of providing a variety of test conditions. The hardware performed nominally and was considered by the investigators to be entirely satisfactory.

INTRODUCTION

The development of special hardware to meet the requirements of the Apollo 16 Microbial Response to Space Environment Experiment (M191) resulted in a very complex and compact piece of experiment flight equipment, the Microbial Ecology Evaluation Device (MEED). The following description will cover the most important components of the MEED flight assembly, the manner in which it was deployed, and the container that was used to transport the experiment hardware from the NASA Lyndon B. Johnson Space Center (JSC) to the NASA John F. Kennedy Space Center and from the recovery ship back to the JSC.

DISCUSSION

Wet Cuvette (Type A) Configuration

The wet cuvettes (fig. 1) were designed to contain exactly 0.05 cm^3 (50 microliters) of microbial suspension. A 25-mm^2 quartz window was provided to allow for exposure to the ultraviolet (uv) components of solar irradiation. A fillport on the side opposite the quartz window was provided to allow adding of the microbial solution just before launch. The cuvette body was constructed of Lexan that had been tinted black to prevent light scatter from adjacent cuvettes. The cuvette body was designed to have a 7° internal slope to prevent possible shadowing of the organisms because of a buildup of tolerances between the Apollo 16 spacecraft computer guidance control system and the cuvettes. The 7° slant was common to the liquid actinometry cuvette assemblies and was implemented throughout the design of the MEED flight assembly.

Dry Cuvette (Types B and C) Configuration

The dry cuvettes (fig. 2) were of a configuration that was slightly different from the configuration of the wet cuvettes. The dry cuvette, which has the same external body dimensions and quartz window as the wet cuvette, provided a means of exposing microorganisms that had been deposited on a 0.45-micrometer Millipore filter chip. The chips were pressed against the quartz window by backing material and a Lexan plug. Sealing wax on the back of the Lexan plug held the microorganisms against the quartz window and prevented the escape of microbes during exposure to space vacuum.

Film Cuvette Configuration

The film cuvette assembly (fig. 3) was identical in size to the dry cuvettes. The film cuvette assembly contained a small piece of Kodak high-resolution film (SO-343) that was secured in place by a black paper gasket, three filter spacers, and a Lexan plug. Sealing wax was used to retain the contents within the cuvette body.

Filters and Filter Pack Assemblies

Provisions were made in the MEED optics system to expose the test systems to the full light of space or to components of the solar uv spectrum. Peak wavelengths of 254, 280, and 300 nanometers were selected by the use of quartz bandpass interference filters. Further optics control was provided by quartz neutral-density filters that controlled the

total radiant energy reaching the exposed microbial test systems; this energy ranged from a minimum of 4×10^{-6} joule/cm^2 (4×10^1 ergs/cm^2) to a full sunlight penetration of 8×10^1 joules/cm^2 (8×10^8 ergs/cm^2). One bandpass filter and five neutral-density filters were combined into one unitized filter pack assembly (fig. 4).

Cuvette Tray Assembly

The cuvette tray provided a means of containment for a maximum of 20 cuvettes or a combination of 16 cuvettes and one temperature recorder (fig. 5). Two styles of cuvette trays were made, but only one style permitted installation of a filter pack assembly. A spring-cushion system capable of withstanding a 100g shock acceleration without component failure was provided. The 100g shock design requirement was imposed consistently throughout the design of MEED flight equipment components to prevent quartz breakage, which could have resulted in a safety hazard to the crewmen during MEED deployment in space.

Temperature Recorders

To minimize the number of variables that could affect the microorganisms contained in the MEED, the flight case assembly was insulated to maintain the temperature at 293° ± 5° K (20° ± 5° C). Compact temperature recorders were designed to measure and record the high and low temperature fluctuations within the MEED trays (fig. 5). A single temperature recorder occupied the volume of four cuvettes and contained a bimetallic coil spring. A scribe attached to the spring recorded the maximum temperature extremes by scratching through carbon black on a smoked glass window. The full effective range of the temperature recorder was 293° ± 10° K (20° ± 10° C).

High-Energy-Particle Dosimeter Case

A high-energy-particle dosimeter case (fig. 6) was designed to replace one of the cuvette trays in each of the three control MEED trays. The case was designed to contain the various dosimeters that measured the tracks of high-energy multicharged (HZE) particles.

The MEED Tray Assembly

All of the previously mentioned components were fitted into five separate MEED tray assemblies (fig. 7). The MEED flight hardware (fig. 8)

consisted of two type A MEED trays that had all-quartz optics systems and one type B control tray that received no solar irradiation and, therefore, contained no optics. The configuration and the content of the type B flight MEED tray were matched exactly by a MEED ground control unit and a MEED vibration control unit. The ground control and vibration control units were shielded from uv irradiation and were insulated for thermal control at 293° ± 5° K (20° ± 5° C). In addition, the vibration control unit was exposed to an anticipated launch vibration profile at exactly the same time as the lift-off of Apollo 16.

The MEED flight assembly that contained three MEED trays was composed of 798 cuvettes that contained microorganisms, 140 neutral-density filters, 28 bandpass filters, eight temperature recorders, one HZE-particle dosimeter, 64 potassium ferrioxalate actinometry cuvettes, 44 photographic film cuvettes, and 18 thermoluminescent dosimetry cuvettes. The microbial systems that were housed in the vented (type C) cuvettes were exposed to the external pressure fluctuations by the use of a venting port in the smaller section of two of the three flight MEED trays.

The MEED Flight Assembly and Deployment

During the Apollo 16 transearth coast extravehicular activity, the MEED hardware was removed from its protective stowage bag in the crew compartment. A 2.54-meter (8 foot 4 inch) tether was attached externally to the MEED flight assembly, the assembly was mounted on the campole, and the hardware was secured to the inside of the spacecraft. The campole and MEED assembly were then installed into a fitting mounted on the opened hatch door of the command module (CM) (fig. 9). A small attitude adjustment of the CM was required to place the appropriate surfaces of the opened MEED assembly directly perpendicular to the rays of the sun. The very slight degree of attitude correction was indicated by a solar-positioning device (sunsight) mounted on the exterior of the MEED (fig. 9). The MEED sunsight contained a pin that, when exposed to the rays of the sun, cast a shadow onto an image-field decal mounted on an inclined plane. A set of 90° crosshairs was scribed on the center of the sunsight decal. These crosshairs marked the point where the top of the pin shadow should lay when the faces of the MEED were exactly perpendicular to the incoming rays of the sun. The placement of the sunlight decal had been determined accurately at the JSC on the sunsight inclined plane by the use of a collimated light source and an accurate positioning table. Reference lines of 7° and 15° and maneuvering instructions were printed on the sunsight decal.

The deployment of the MEED in deep space exposed the microbial systems to a potential thermal problem. A black and white thermal coating pattern was painted on the cover of the MEED assembly (fig. 10)

to provide the proper thermal control after deployment and before opening the MEED. The proportioning of black and white paint was to provide the proper internal temperature preconditioning inside the MEED. To meet the requirement for thermal balance within all portions of the MEED, the device was designed to remain within the 293° ± 5° K (20° ± 5° C) temperature range after deployment. The position of the flight temperature recorders is shown in figure 11. Results from the MEED flight temperature recorders are shown in table I. The average low temperature was 2° K (2° C) below the optimum temperature of 293° K (20° C), and the average high temperature was 7° K (7° C) above the optimum; all high and low temperature readings were similar. The full sunlight sections of the assembly did not sustain higher temperatures than did other areas of the MEED flight assembly; therefore, 10 minutes of full sunlight exposure did not upset the thermal balance of microbial systems in those sections. Whereas the MEED temperature specifications called for 293° ± 5° K (20° ± 5° C), the actual performance was an average temperature of 295° ± 5° K (22° ± 5° C). The actual maximum temperature recorded was 3° K (3° C) above the maximum specified but was still within an acceptable range. Maintaining this temperature range is considered excellent performance for a passive system operating for 18 days without internal thermal control.

The MEED Stowage Bag

The MEED stowage bag absorbed the launch vibrations and provided thermal insulation for MEED flight equipment. The bag was made from nonflammable Beta cloth and nonflammable Fluorel sponge foam and weighed 772 grams (1.7 pounds).

Biological Experiments Transporter

The nonflight temperature of the microbial systems in the MEED had to be maintained at 293° ± 5° K (20° ± 5° C). This temperature restraint required the use of an insulated transporter that was designed to provide passive control of the temperature within the specified range (fig. 12). The transporter contained the MEED, the biostack experiment, the ground control MEED tray, a heat sink, and a 7-day temperature recorder (fig. 12). An additional means of determining internal container temperature was provided by a remote temperature-indicating device. The transporter was approximately the size of a cube with 48.26-centimeter (19 inch) sides and weighed approximately 27.2 kilograms (60 pounds) when fully loaded.

CONCLUDING REMARKS

The Microbial Ecology Evaluation Device met the requirements of the Microbial Response to Space Environment Experiment (M191) system; all systems operated as designed. Only one anomaly occurred during operation of the unit. After the 10-minute exposure period, the Apollo 16 command module pilot had some difficulty in locking the flight hardware cover in the closed position. By keeping the Microbial Ecology Evaluation Device cover closed until it could be securely latched inside the spacecraft, any possible degradation to the experiment was avoided. A similar problem had occurred in a vacuum chamber during the Microbial Ecology Evaluation Device qualification test program. To solve this problem, the latching device was redesigned. The possibility of the light seals expanding inside the Microbial Ecology Evaluation Device and an unforeseen human engineering problem prevented immediate securing of the cover by the Apollo 16 commander. Even with the cover closure problem, the science investigators associated with the project consider the performance of the Microbial Ecology Evaluation Device highly satisfactory. From the engineering viewpoint, the performance of the hardware was excellent.

TABLE I.- POSTFLIGHT TEMPERATURE READINGS FROM THE MEED

Temperature recorder serial number	High temperature			Low temperature		
	°K	°C	°F	°K	°C	°F
9	301	28	81	291	18	64
10	300	27	80	290	17	63
12	300	27	80	292	19	66
13	300	27	80	290	17	63
15	300	27	80	292	19	66
16	302	28	82	288	15	59
18	300	27	80	290	17	63
19	298	25	77	291	18	64

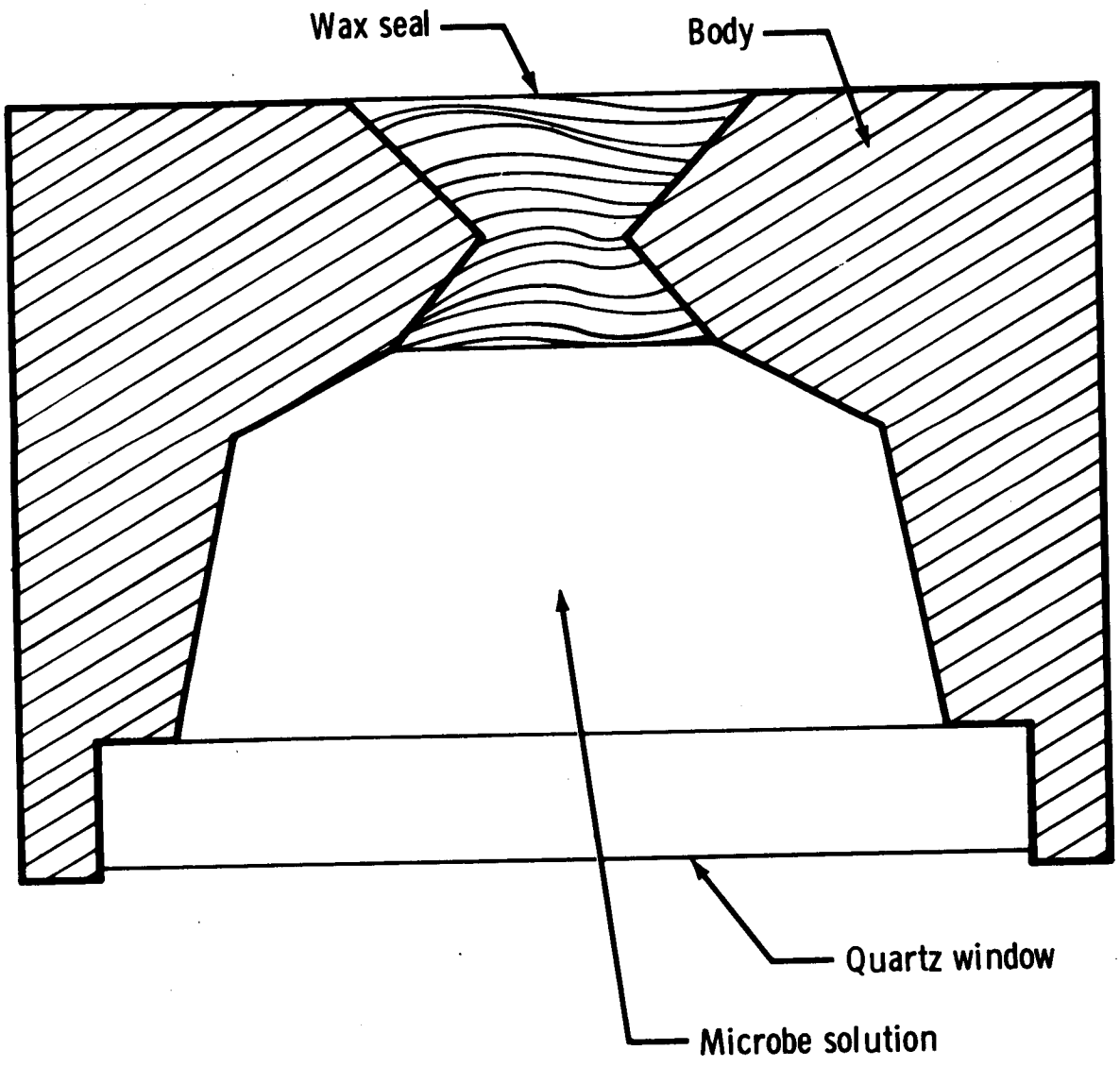


Figure 1.- Wet cuvette (type A) configuration.

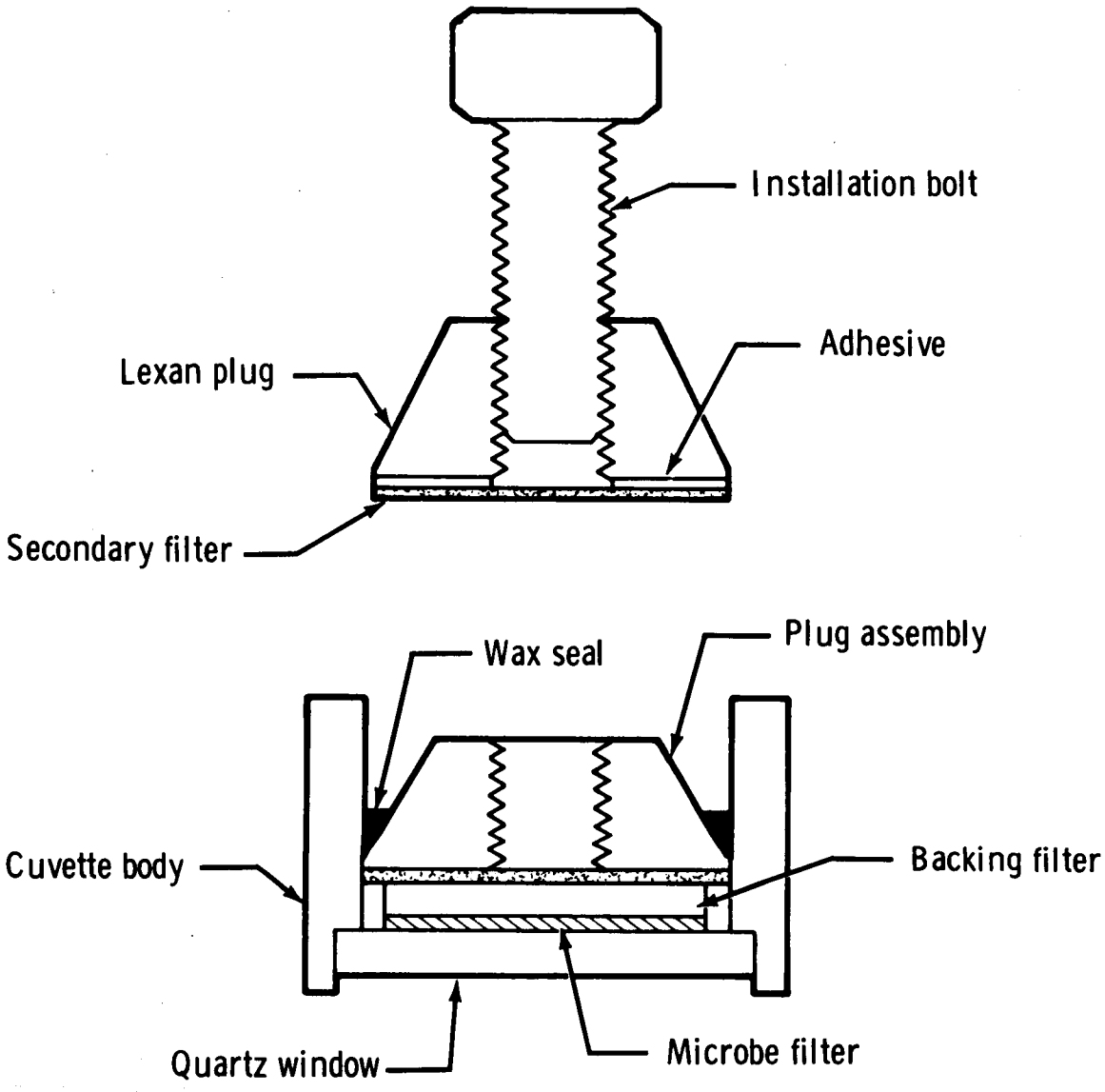


Figure 2.- Dry cuvette (types B and C) configuration.

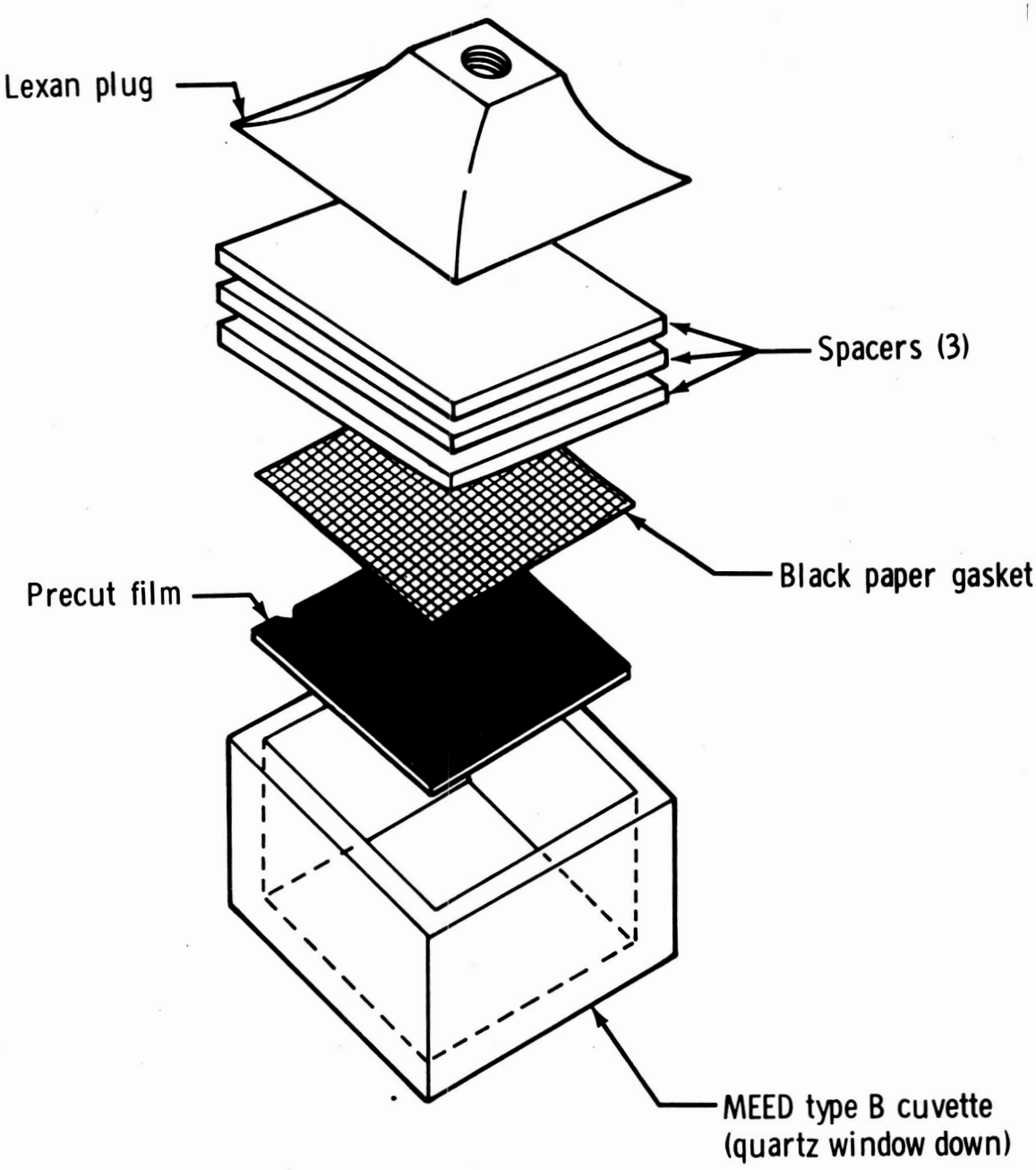


Figure 3.- Film cuvette assembly.

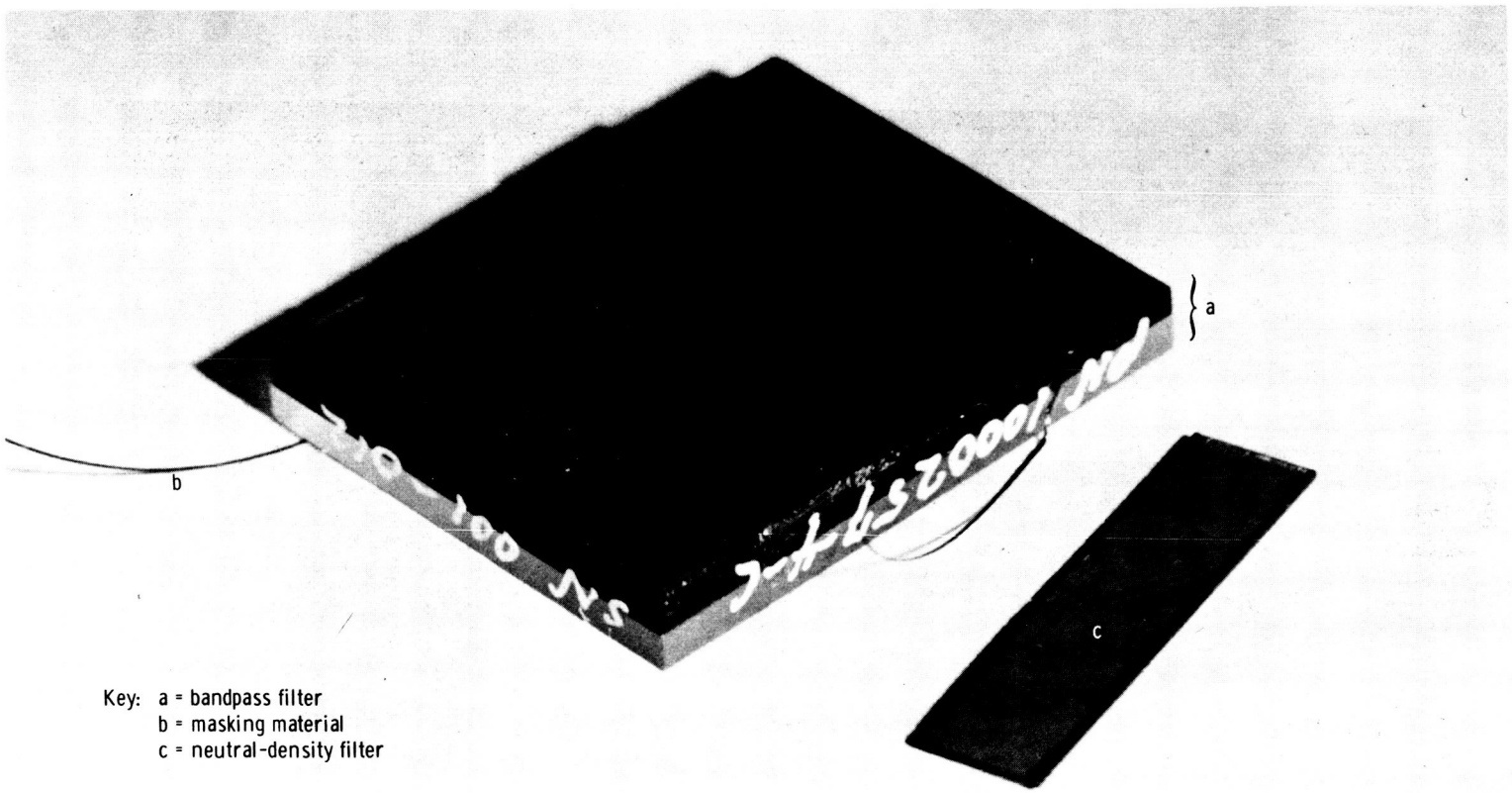
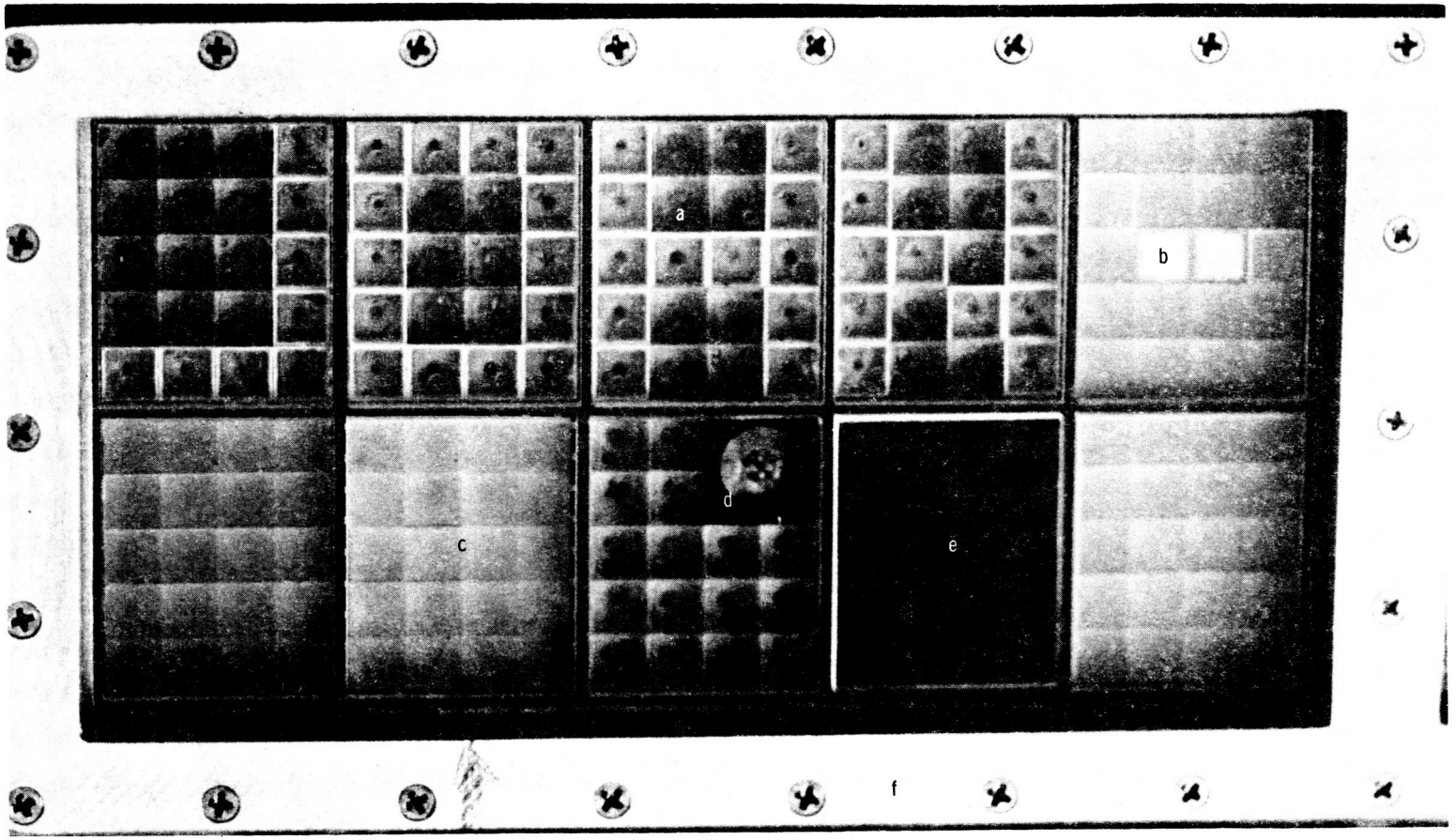


Figure 4.- Optical filter pack assembly (partially assembled).



Key: a = wet cuvette (type A)
b = dry cuvette (type B or C)
c = cuvette tray
d = temperature recorder
e = bandpass filter
f = MEED tray

Figure 5.- Portion of MEED tray.

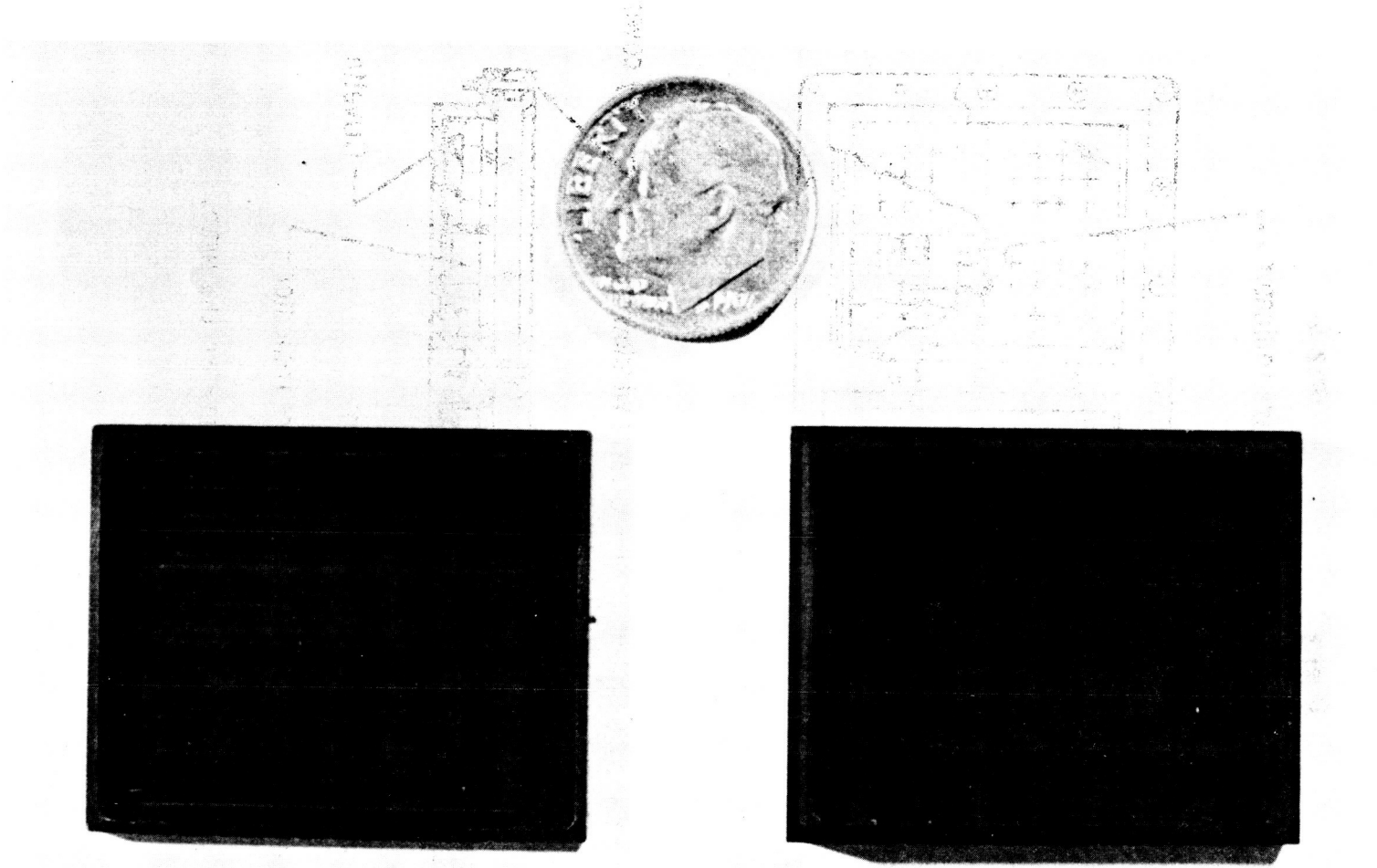


Figure 6.- High-energy-particle dosimeter case.

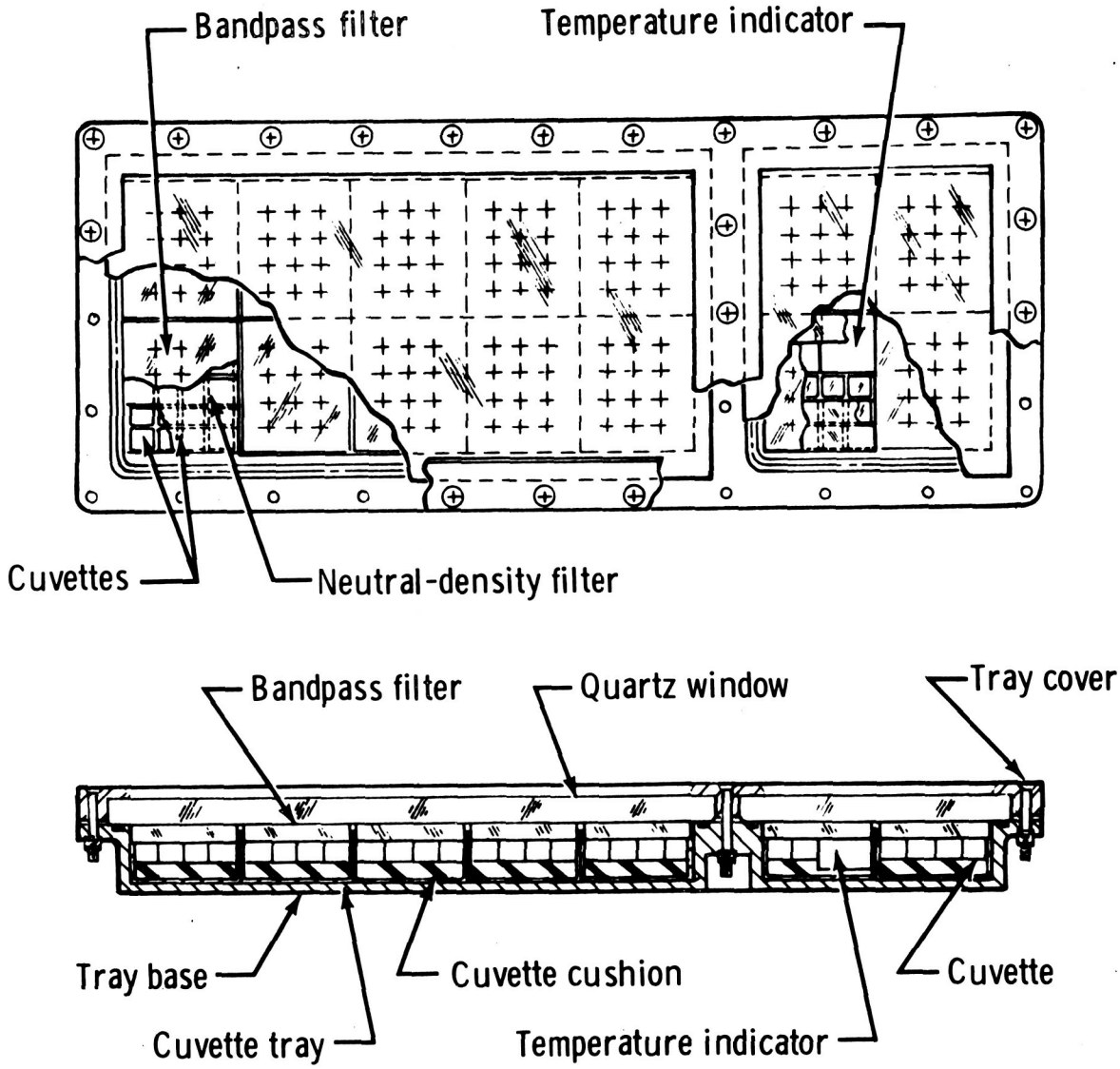


Figure 7.- Tray interior details of the MEED.

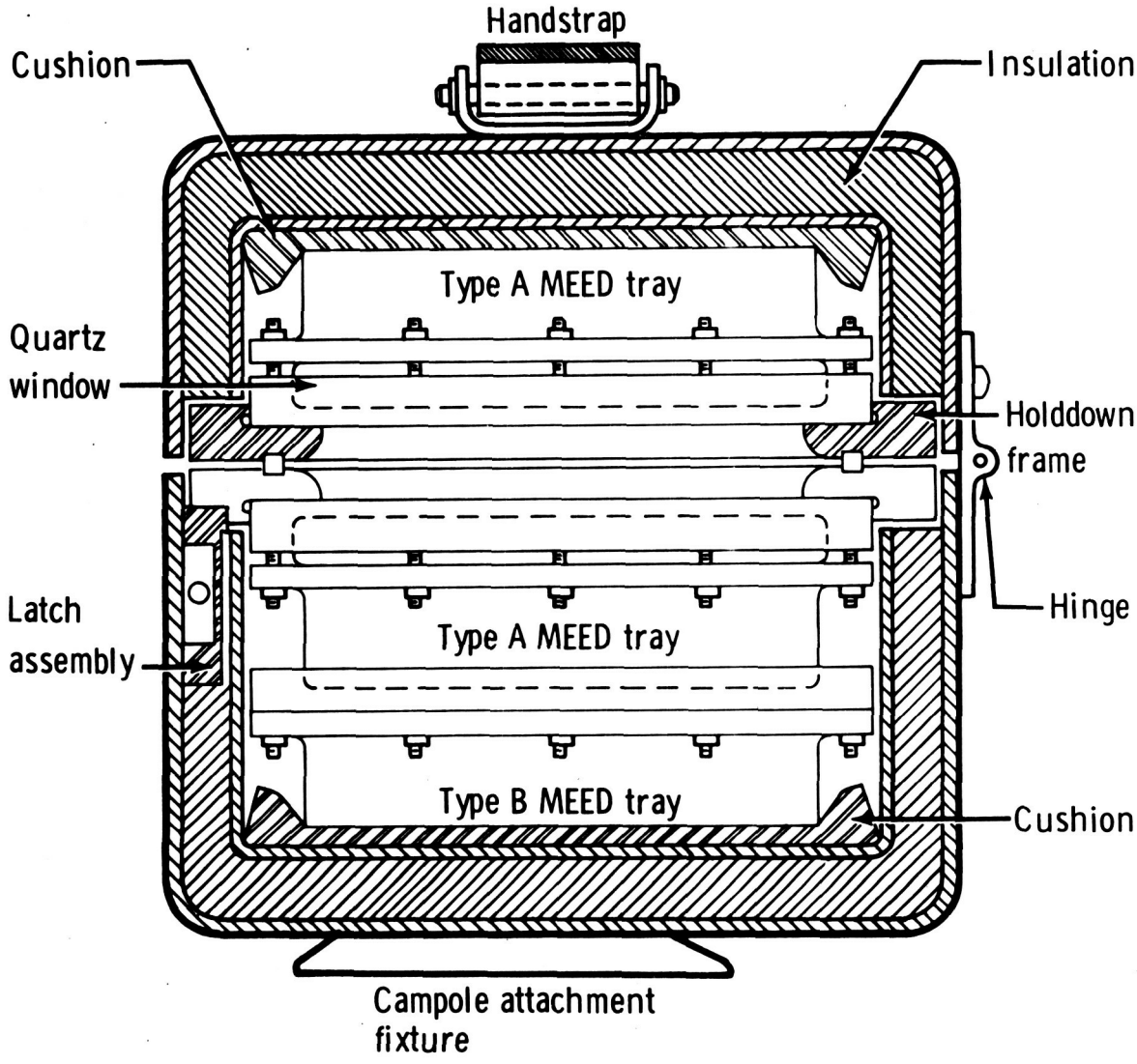


Figure 8.- Interior details of MEED flight assembly (end view).

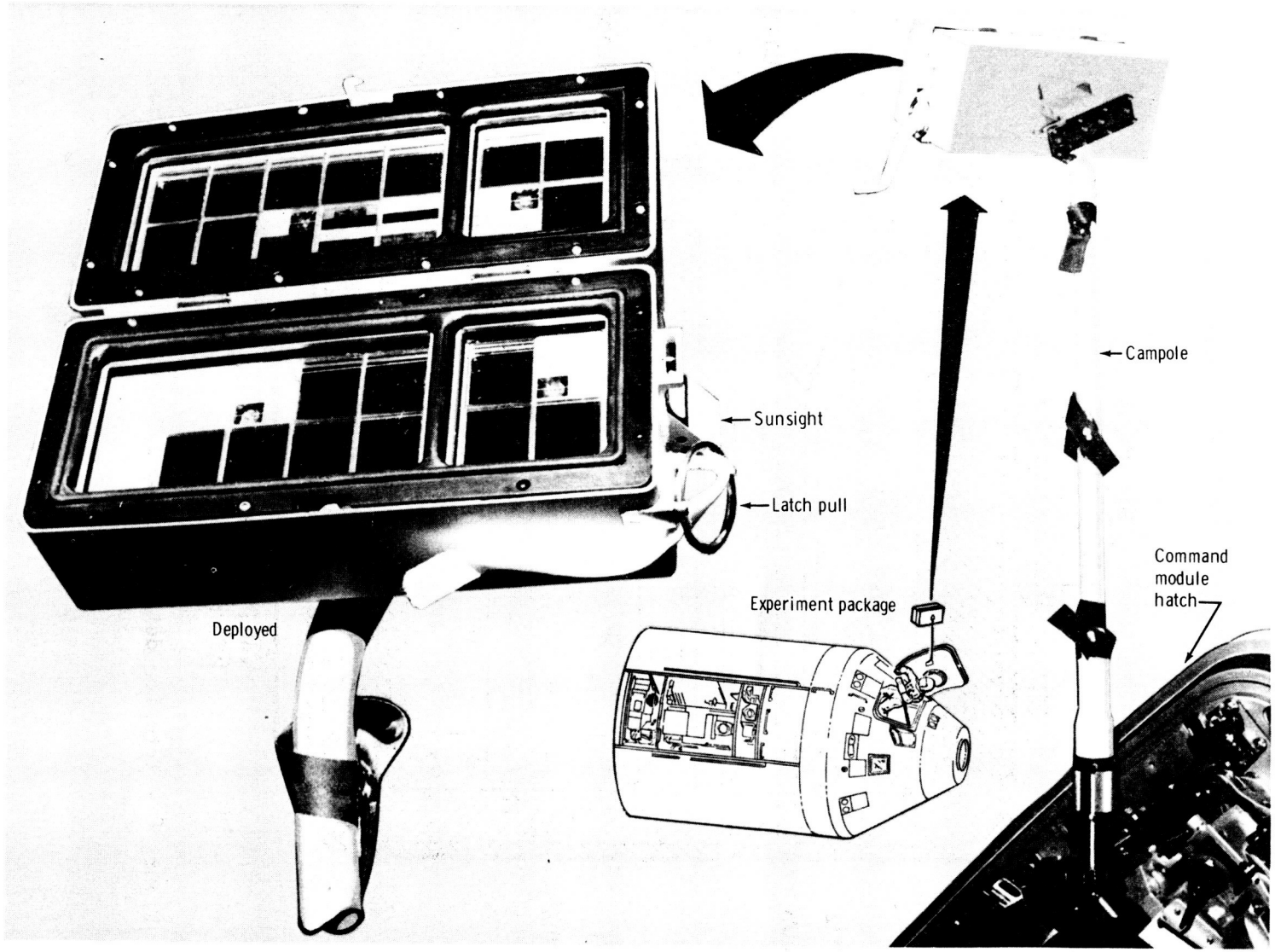


Figure 9.- Hardware for the Microbial Response to Space Environment Experiment (M191).

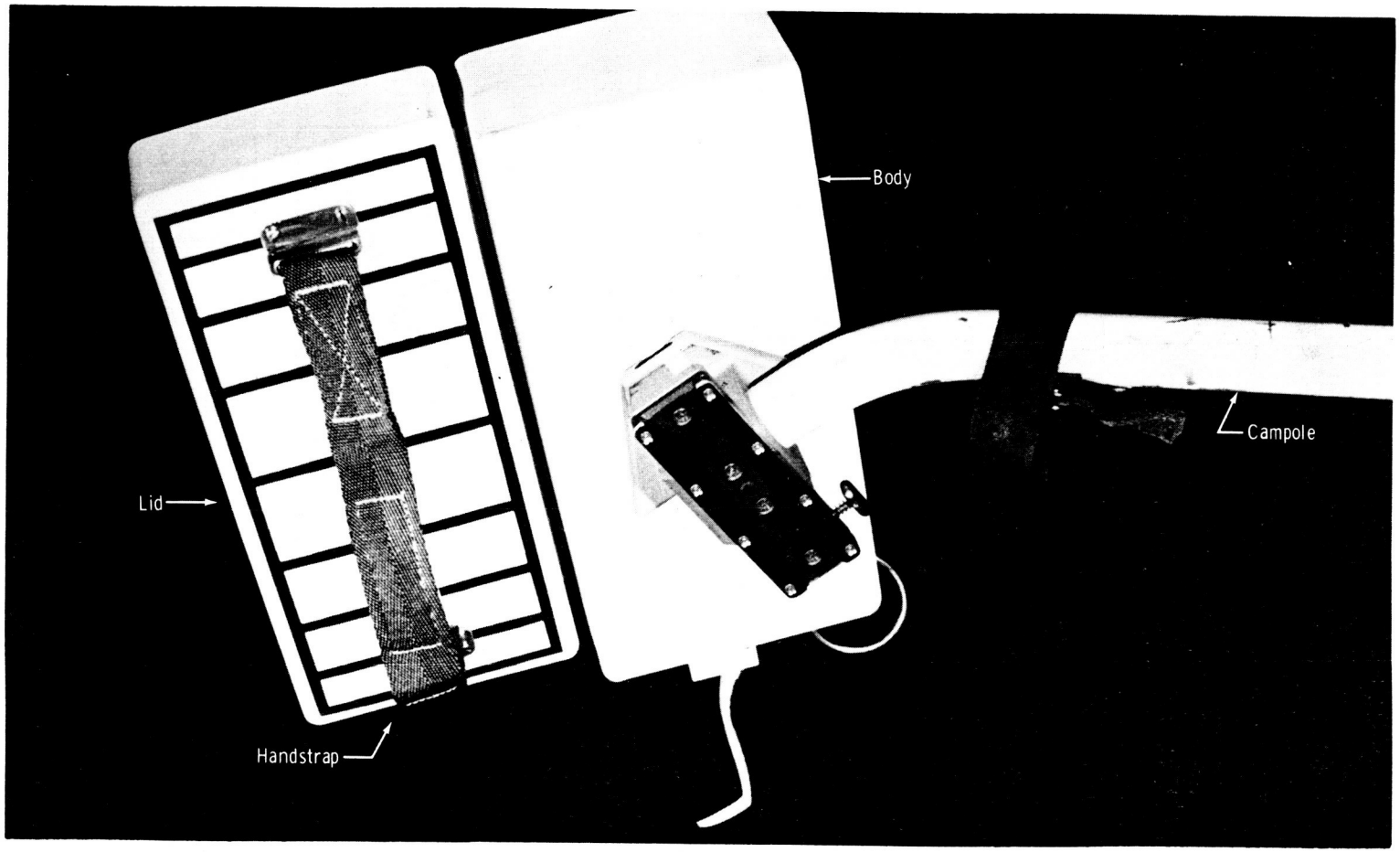


Figure 10.- Reverse side of opened MEED showing thermal coating pattern on lid.

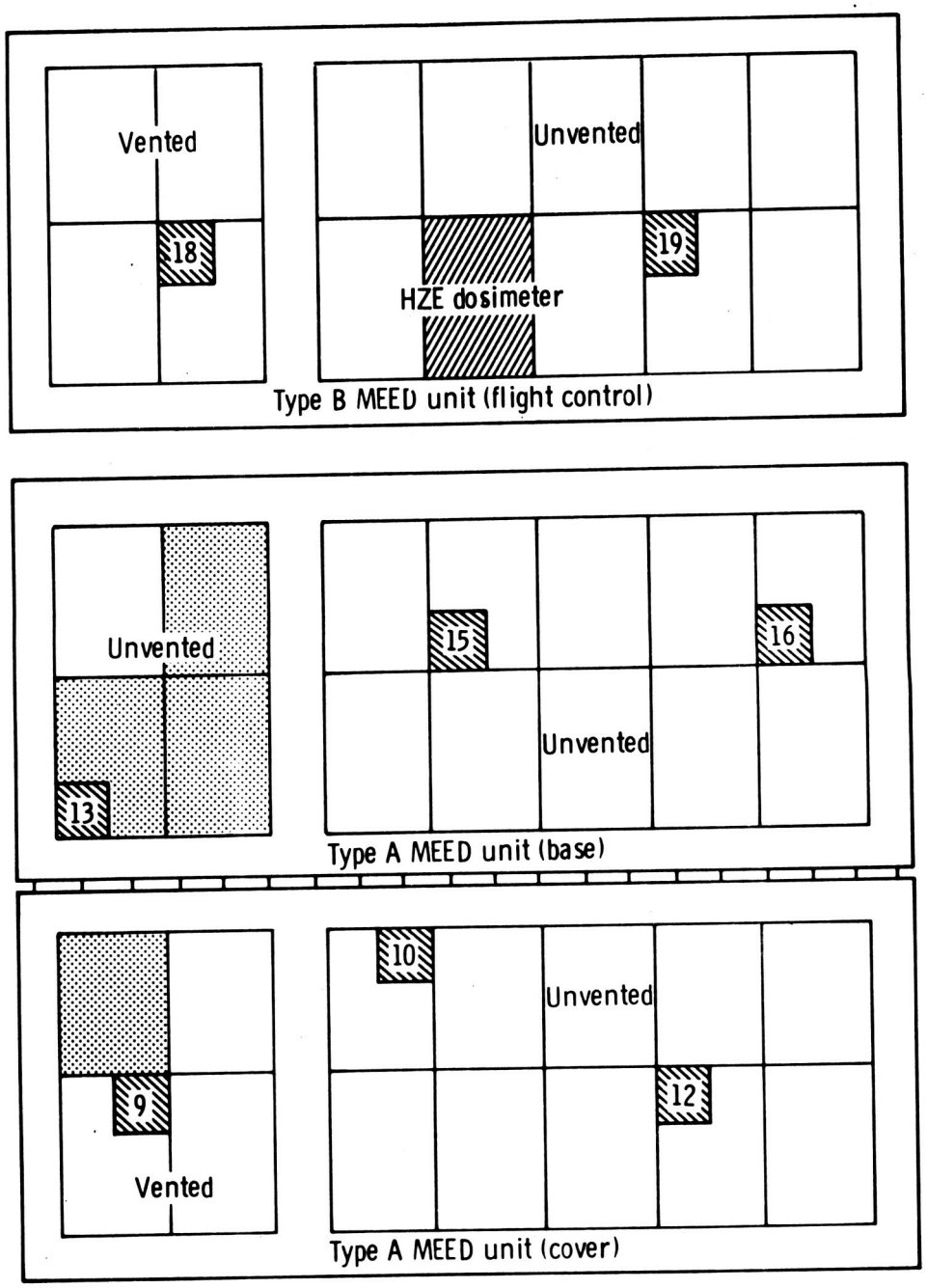


Figure 11.- Positions of flight temperature recorders in MEED flight assembly.

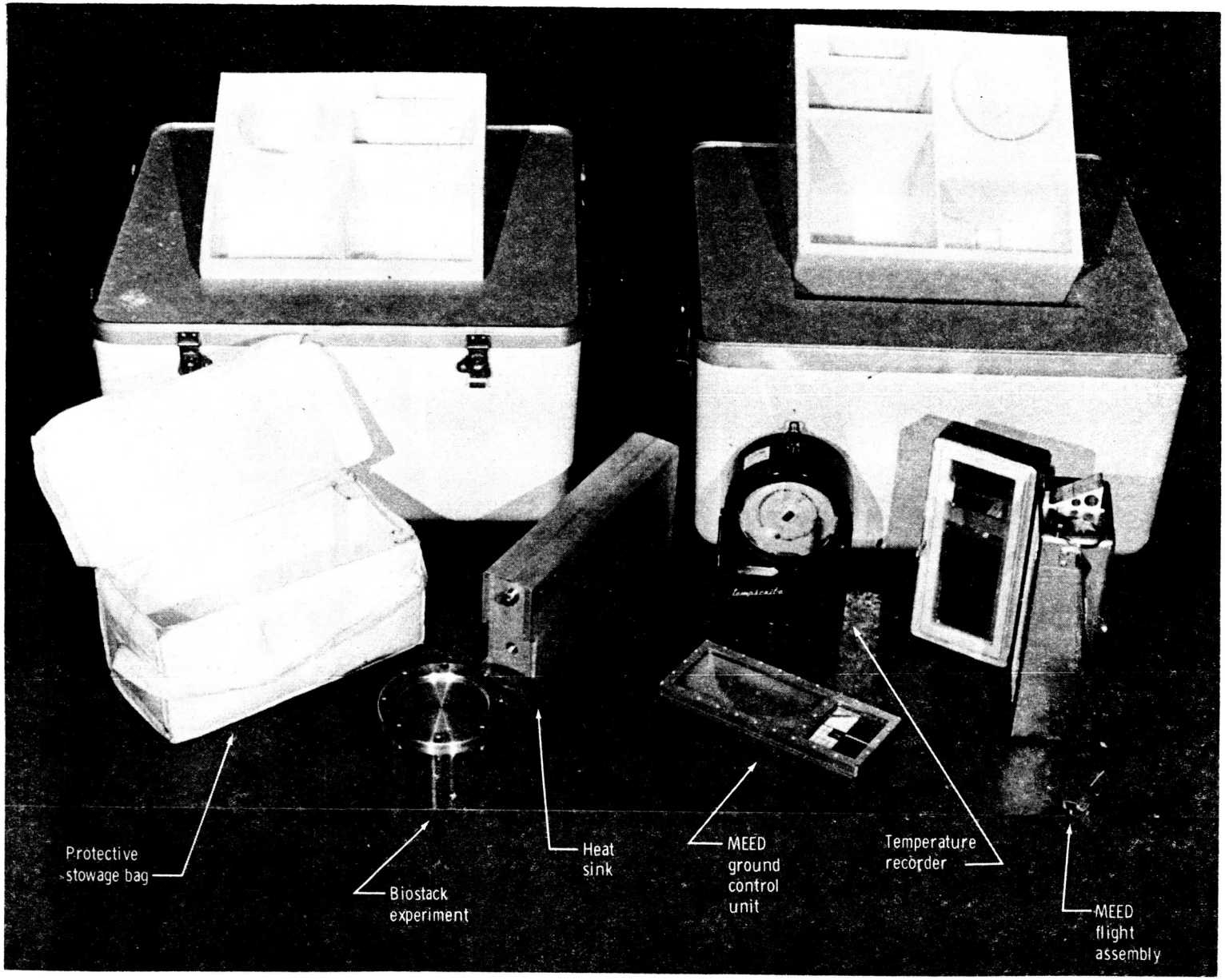


Figure 12.- Internal components of biological experiments transporter.

CUVETTE LOADING AND UNLOADING PROCEDURES

By Carolyn Carmichael* and Walter L. Ellis*

ABSTRACT

The cuvettes for the flight hardware used in the Microbial Response to Space Environment Experiment (M191) were loaded with the biological test systems according to the methods described. After the flight, the experiment package was returned to the NASA Lyndon B. Johnson Space Center and dismantled. Then, the individual cuvettes were removed from the hardware and unloaded according to the procedures described.

SUMMARY

Biological test systems were included in the Microbial Response to Space Environment Experiment (M191). These systems were represented in both wet and dry conditions and were contained in small chambers (cuvettes). Procedures for the loading and unloading of the wet (type A) and dry (types B and C) cuvettes were successfully accomplished.

INTRODUCTION

The biological test systems for Apollo 16 Microbial Response to Space Environment Experiment (M191) were contained in cuvettes within the flight hardware. General operating procedures were used in the loading and unloading of the wet and dry cuvettes. Specific ground support equipment was designed for these functions and was used when necessary. In some cases, special techniques and equipment were required for specific areas being studied.

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*Northrop Services, Inc., Houston, Texas.

DISCUSSION

Wet-Cuvette Loading Procedures

The wet, or type A cuvettes, (fig. 1) were designed to contain 0.05 cm^3 (50 microliters) of a fluid suspension of microorganisms. The small volume of these cuvettes necessitated the use of a special apparatus that would allow accurate aseptic filling and that would minimize bubbles. This wet-cuvette loader (fig. 2) consisted of an electric turntable/cuvette holder and a piston-mounted needle equipped with a fill hose connected to a repipette that was equipped with a lambda dial for adjusting the fill volume to 0.05 cm^3 (50 microliters). After the cuvette was placed in the turntable by the use of sterile forceps, the filling process was initiated. The turntable and the barrel were activated simultaneously by a double toggle switch. Thus, with the turntable spinning, the barrel came down, inserting the needle into the orifice of the cuvette. The cuvette holder continued to spin during filling to force fluid into the corners of the cuvette and to force the air out. This continuous spinning reduced the number of bubbles in the fluid. Filling was completed by activating the repipette plunger once to deliver the inoculum. The barrel was retracted, and the turntable was stopped by turning off the control switch.

The filled cuvette was sealed with a heat-sealing tool (fig. 3). The tip of the sealing tool was held flush on the inside slope of the cuvette hole. Then, the sealing wax was touched to the hot wire, allowing the wax to melt and to flow down the needle, across the fluid interface, and up the other side (fig. 1). During the sealing process, the turntable was rotated manually to ensure an even fill. After the wax had cooled, the excess was removed from the exterior of the cuvette by the use of a sterile scraping tool. Each cuvette was inspected visually to ensure proper filling and sealing. The serial number was recorded, and the cuvette was placed in a holding container.

Dry-Cuvette Loading Procedures

The dry cuvettes were designated types B and C. The type was determined by whether the cuvette was unvented (type B) or was vented to the atmosphere (type C). A known concentration of an organism suspension was filtered through a sterile 47-millimeter gridded, white Millipore filter pad that had a mean pore diameter of 0.45 micrometers. The pad was removed by the use of sterile forceps, was placed on sterile Whatman number 1 filter paper in a sterile Petri dish, and was blotted free from fluid. The Millipore filter pad was placed in a sterile filter

punch (grid side up). This punch was designed to cut chips the size of the interior of the cuvette. The pad was always handled with sterile forceps. Care was taken to ensure that each chip was punched the same distance from the periphery of the pad to avoid possible variation in microbial concentration. The filter chips were punched one at a time and were placed in a dry cuvette that had already been placed in the holding fixture (fig. 4). Seven to nine chips were punched from each pad. The Millipore filter chip was placed in the cuvette so that the grid side of the chip was against the inside of the cuvette window. Two sterile spacers were inserted behind the Millipore filter chip. A sterile plug assembly, consisting of a screw set into a pyramid, was inserted into the cuvette.

The external surface of the plug assembly was sealed as described previously. The type B cuvettes were sealed completely by removing the screw from the plug and filling the screw hole with wax. The screw hole was left unsealed on the type C cuvettes to provide the vent to the surrounding atmosphere. All cuvettes were inspected for proper seal and cleanliness. The serial numbers were recorded, and the cuvettes were placed in a holding container.

Composition Tray Loading

Composing trays representing the three flight hardware trays and the two nonflight control trays were filled with the loaded cuvettes. Each cuvette was placed in a composing tray in a position corresponding to its ultimate position in the experiment hardware. Subsequently, the composing trays containing the cuvettes were moved to a darkroom in which the actinometry cuvettes were added. Then, all cuvettes were loaded into the experiment hardware under red-light conditions.

Hardware Disassembly

After the Apollo 16 flight, the experiment hardware was returned to the NASA Lyndon B. Johnson Space Center and dismantled. Red-light conditions were maintained until the actinometry cuvettes were removed and stored in a light-tight container. The remaining cuvettes were placed in the composing trays in their exact preflight position, then distributed to the separate investigators.

Unloading of Wet Cuvettes

For the unloading, each type A cuvette was placed in a holder with the wax plug up. A 1.5-millimeter (1/16 inch) sterile drill bit was used to ream the sealing wax from the cuvette hole. Then, each investigator extracted the fluid according to his own methods.

In general, the individual methods for extracting the fluid were variations of the following procedure. A 0.2-cm^3 (0.2 milliliter) aliquot was withdrawn from a 1.0-cm^3 (1.0 milliliter) diluent tube by the use of a syringe and a 26-gage needle. The needle was placed into the orifice of the cuvette, and as much cuvette fluid as possible was withdrawn. The fluid in the syringe was ejected into an empty, sterile vial. The same syringe was used to withdraw another 0.2-cm^3 (0.2 milliliter) aliquot from the original diluent tube. The second aliquot was used to flush the cuvette by alternate injection and withdrawal of fluid. The fluid was ejected into the same tube used for the first aspiration; this procedure was repeated once. Then, the syringe was flushed with the remaining fluid in the original diluent tube. The syringe wash fluid was combined with the cuvette wash fluid. The plating techniques varied among investigators.

Unloading of Dry Cuvettes

Each type B and C cuvette was placed in a holder with the pyramid up. A pyramid-extracting tool was screwed into the hole, and the pyramid was pulled out. The wax left in the cuvette was scraped out by the use of a sterile scraping tool. The two spacers were removed with sterile forceps. Finally, the Millipore filter chip was removed with sterile forceps and was processed according to the methods of the individual investigator. A typical method was as follows. The Millipore filter chip was placed in a small, sterile vial. The cuvette window was washed with 1.0 cm^3 (1.0 milliliter) of sterile distilled water, and the wash fluids were added to the vial containing the filter chip. The filter chip was soaked in the distilled water for 24 hours at ambient temperature to facilitate the removal of spores. Then, each vial was vortexed for 1 minute. The plating techniques varied among investigators.

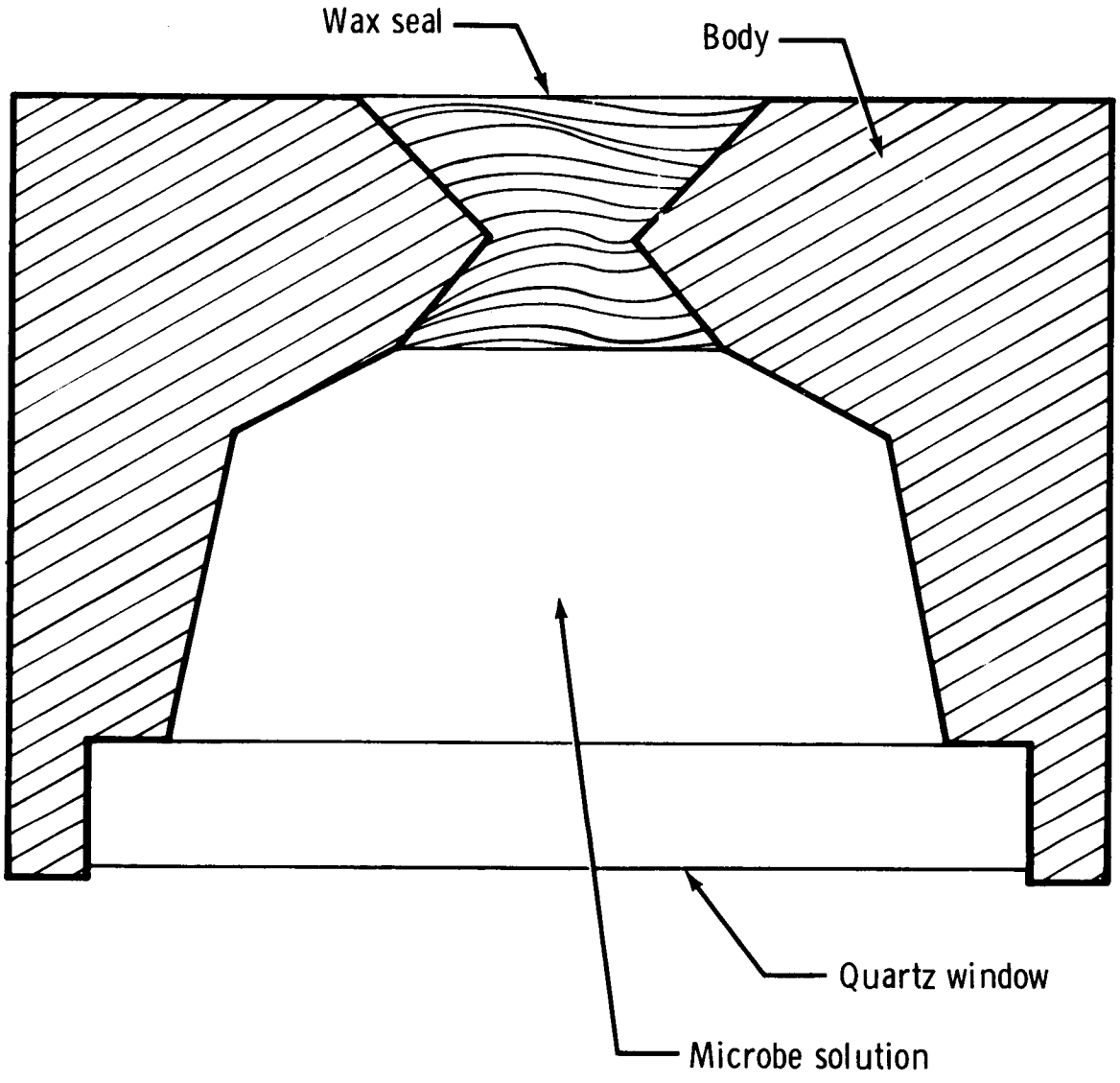

Figure 1.- Wet-cuvette configuration.

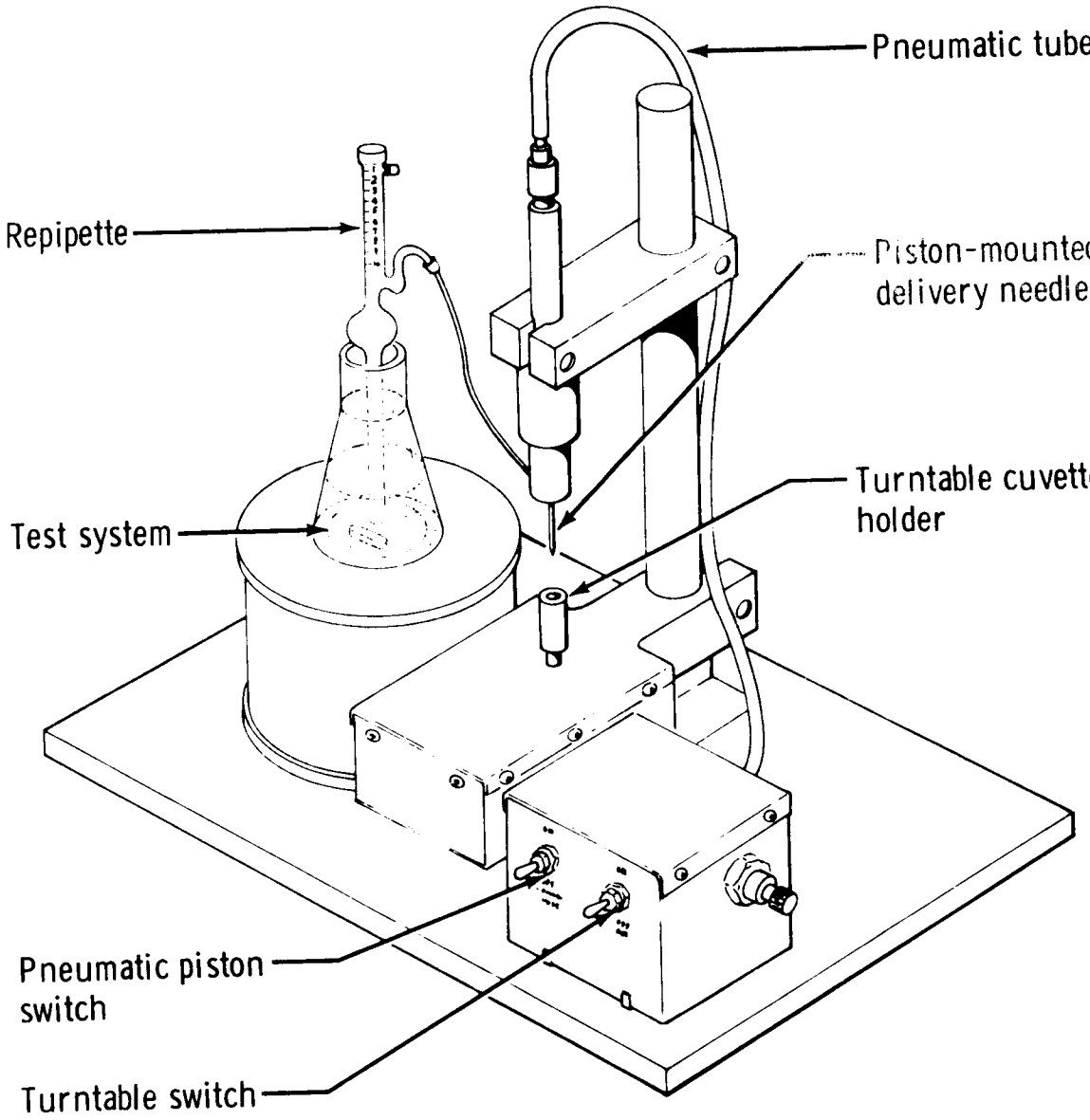

Figure 2.- Wet-cuvette filling station.

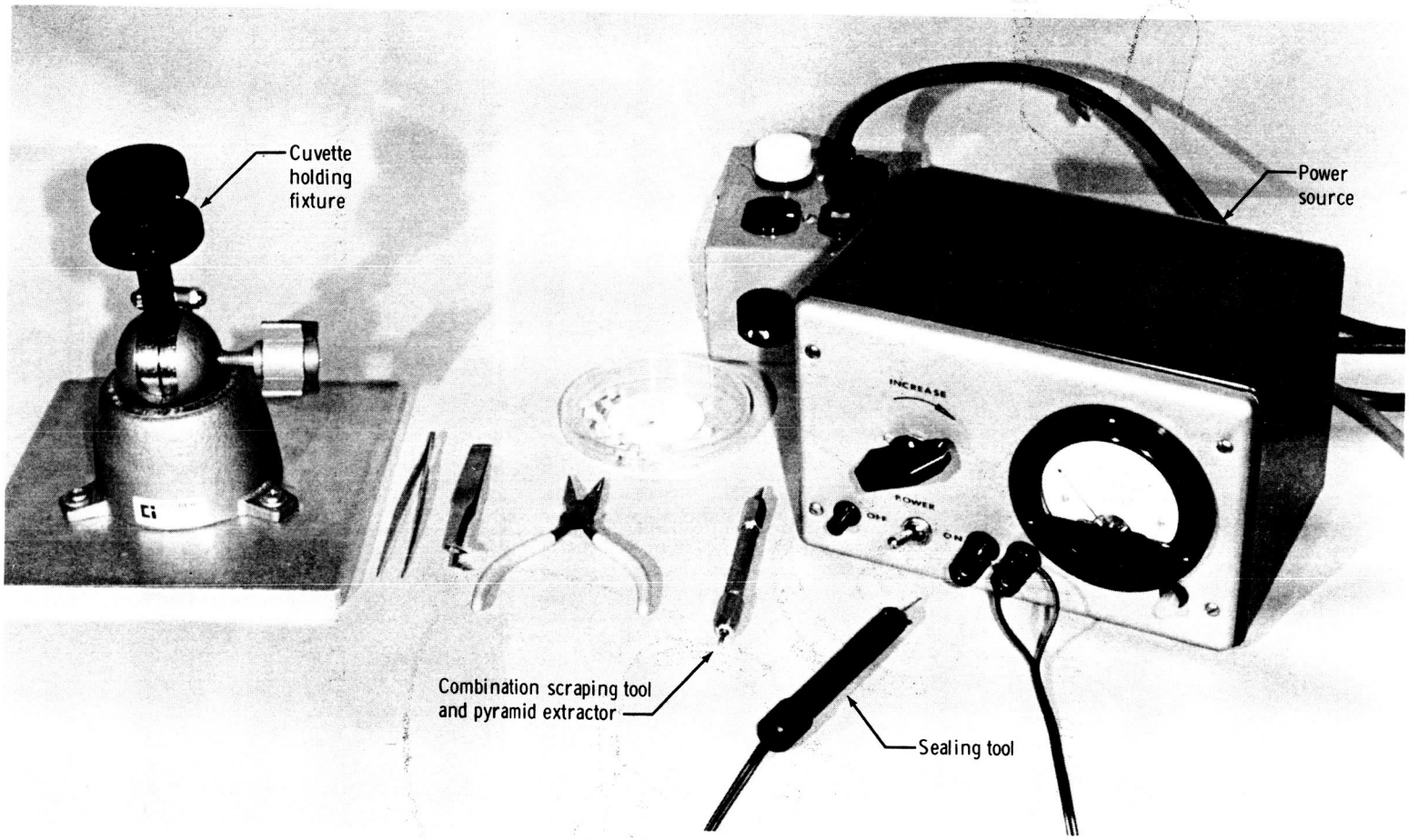

Figure 3.- Heat-sealing apparatus.

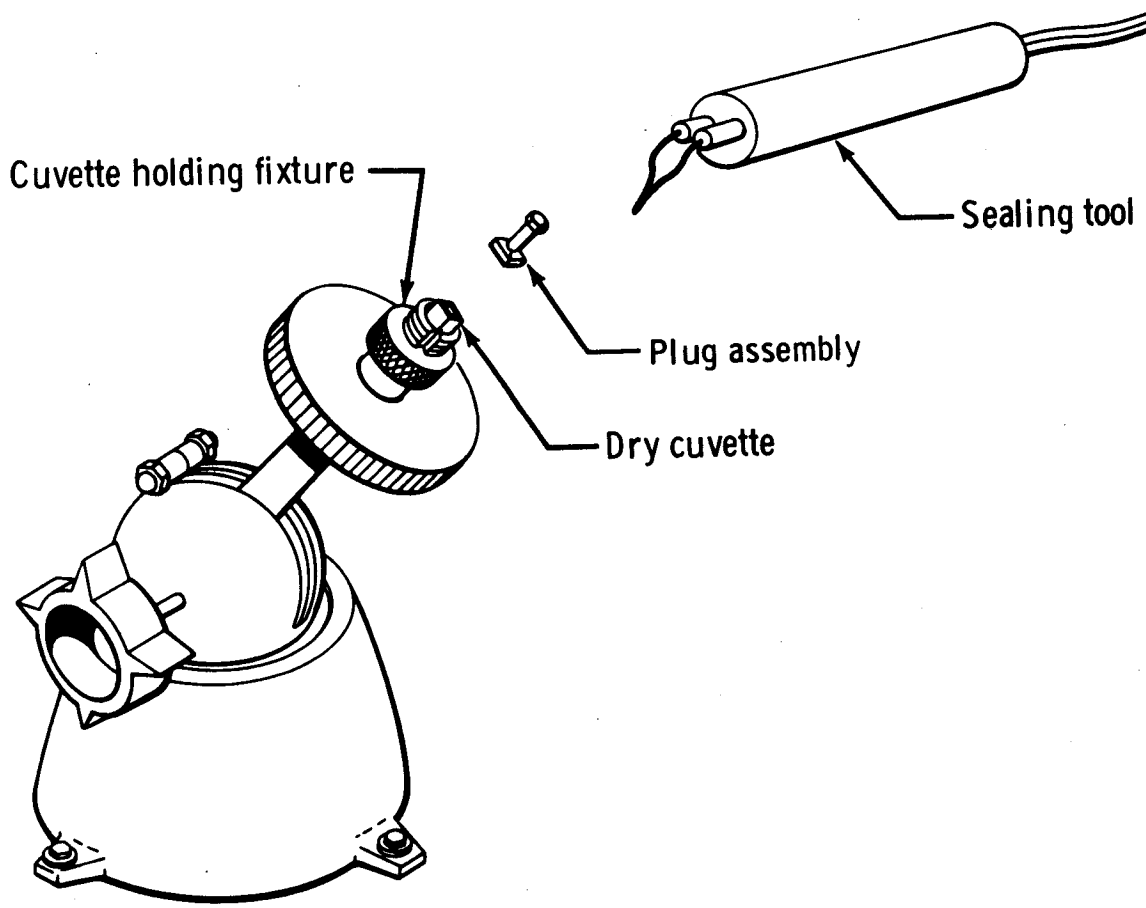

Figure 4.- Dry-cuvette plug installation.

SECTION II BIOLOGICAL TESTS CONDUCTED

INFECTIVITY AND EGG PRODUCTION OF *NEMATOSPIROIDES DUBIUS* AS AFFECTED BY SPACE FLIGHT AND ULTRAVIOLET IRRADIATION

By Richard A. Long,* Walter L. Ellis,* and
Gerald R. Taylor
Lyndon B. Johnson Space Center

ABSTRACT

Nematospiroides dubius was tested to determine the infective potential of the third stage larvae and the egg-production and egg-viability rates of the resulting adults after they are exposed to space flight and solar ultraviolet irradiation. The results are indicative that space-flown larvae exposed to solar ultraviolet irradiation were rendered noninfective in C_{57} mice, whereas flight control larvae that received no solar ultraviolet irradiation matured at the same rate as the ground control larvae. However, depressed egg viability was evident in the flight control larvae.

SUMMARY

Space flight and solar ultraviolet irradiation had a significant effect on the intestinal parasite *Nematospiroides dubius* in its larval stage. Ultraviolet irradiation in the space environment rendered this organism incapable of completing its natural life cycle. Moreover, the control group that was exposed to space but that received no solar irradiation also had a reproductive failure. That extraterrestrial flight alone was capable of inducing a reproductive deficiency in this complex, multicellular organism is a possibility.

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*Northrop Services, Inc. Houston, Texas.

INTRODUCTION

The role of solar irradiation of biological systems in a space environment is a new and challenging discipline. Early testing of space-exposed organisms was indicative that filtered ultraviolet (uv) irradiation caused almost complete inactivation in some species and that this irradiation caused an increased viability in other species (ref. 1). Laboratory investigations of the mutagenic effects of selected portions of the uv spectrum have been reported (ref. 2). Although most investigations of radiation effects have been restricted to bacteria and protozoa, X-irradiation and gamma irradiation have been shown to inhibit normal development in the eggs of some parasitic nematodes. Nematospiroides dubius is a common intestinal parasite of several species of feral rodents and was selected for testing because of its multicellular structure, complex reproductive system, and relative ease of culture and maintenance in the test environment. The purpose of this investigation was to determine the effects of space flight and solar uv irradiation on the infectivity of the larval stage of Nematospiroides dubius, and to evaluate the reproductive function in the resulting adults.

METHODS

Filariform larvae of Nematospiroides dubius were used to infect a colony of male C_{57} mice to establish a reservoir of potentially infective larvae. Eighteen days after inoculation, eggs were noted in the feces (fig. 1). Fecal pellets from the infected mice were collected on moist paper towels and were emulsified in deionized water. The emulsion was filtered through three-ply surgical gauze and centrifuged at 250 × g for 20 minutes. The supernatant was discarded, the sediment was suspended in 2 to 3 cm^3 (2 to 3 milliliters) of distilled water, and 1 cm^3 (1 milliliter) of the suspension was transferred by use of a Pasteur pipette onto moist filter paper in individual 20- by 100-millimeter Petri plates.

The cultures were incubated at 298° K (25° C) for 5 days, after which infective larvae were noted at the perimeter of the filter paper (fig. 2). The larvae were washed from the culture plates with warm distilled water into a modified Baermann filtering device. The filtered larval suspension was collected in 15-cm^3 (15 milliliter) conical tubes, centrifuged at 250 × g for 2 minutes, and washed once to clear particulate matter. The supernatant was discarded, and the larvae were transferred to Virtis vials containing 5 cm^3 (5 milliliters) of water for

storage. The number of larvae in suspension was estimated by dilution count, and the total volume was adjusted to 4000 larvae/cm^3 (4000 larvae/ml).

Each of the 92 type A cuvettes was filled 16 days before launch with the aid of a 0.05-cm^3 (50 microliter) syringe containing approximately 200 larvae in suspension. The cuvettes were sealed and categorized as 32 flight experimental (16 uv_1 and 16 uv_2), 20 flight control (FC), 20 vibration control (VC), and 20 ground control (GC) cuvettes in accordance with the experiment design outlined in figure 3. The 32 flight experimental cuvettes were positioned in the Microbial Ecology Evaluation Device (MEED) trays under selected neutral-density filters. The 20 flight control cuvettes were positioned in the trays to prevent solar uv irradiation. The MEED was transported to the NASA John F. Kennedy Space Center and loaded on board the Apollo 16 command module. The ground and vibration control cuvettes were loaded into assemblies identical to the flight unit. The ground control unit remained in the proximity of the flight experimental package until launch. The vibration control unit was tested at the experimental dynamics laboratory at the NASA Lyndon B. Johnson Space Center (JSC) to simulate launch vibration. This test was conducted simultaneously with the Apollo 16 launch, and the vibration control unit was returned to the JSC Lunar Receiving Laboratory for the remainder of the test period.

In 14 days of space flight, the larvae were exposed to zero gravity, fluctuating magnetic fields, high mass ionizing radiation, and a 10-minute exposure to filtered solar irradiation at a wavelength of 254 nanometers. The cuvettes were returned to the laboratory 5 days after splashdown. A 1.5-millimeter (1/16 inch) drill bit was used to remove the wax seal of each cuvette. The larval suspension was removed by the use of a 0.5-cm^3 (0.5 milliliter) syringe and 26-gage needle, and this suspension was transferred to the well of a spot plate containing 0.2 cm^3 (0.2 milliliter) of distilled water. Each cuvette was aspirated with an additional 0.05 cm^3 (0.05 milliliter) of distilled water to ensure the removal of all larvae. The suspension volume was divided equally in two individual wells of the plate to yield 75 to 100 larvae per aliquot. Larvae in each aliquot were counted with the aid of a dissecting microscope and were inoculated per os into individual male C_{57} mice. All mice were housed in individual cages for an infection period of 28 days. On postinoculation day 22, fecal pellets were examined quantitatively by the use of the Stoll technique (ref. 3) for eggs and were subcultured for viability. On postinoculation day 28, all mice were killed by cervical dislocation. Ten centimeters of the proximal portion of the small intestine, including the pyloric sphincter, were removed from each

mouse by use of the method prescribed by Haley (ref. 4), and were examined to detect the presence of adult worms (fig. 4).

RESULTS

From the ground control larvae that had remained with the flight hardware at all times except during the flight, certain information was derived (fig. 5). The mean inoculum size for the ground control group was approximately 90 larvae per mouse, and each mouse yielded approximately 40 adults (30 of which were female) at autopsy. Each female laid an average of 760 eggs per day; 20 percent of these eggs were viable.

Similar data are presented in figure 5 for the vibration control larvae that remained at the JSC and that were vibrated at the time of the Apollo 16 launch. The mean inoculum size for the vibration control group was approximately 95 larvae per mouse; each mouse yielded approximately 43 adults (25 of which were female) at autopsy. Each female laid an average of 850 eggs per day; 20 percent of these eggs were viable.

The flight control group consisted of larvae that were flown in the flight hardware but were never exposed to uv irradiation. The mean inoculum size for the flight control group was approximately 90 larvae per mouse (fig. 5), and each mouse yielded approximately 45 adults (approximately 28 were female) at autopsy. Each female laid an average of 670 eggs per day; only 8 percent of these eggs were viable.

The resulting counts of larvae exposed to uv irradiation during space flight are shown in figure 6. The mean inoculum size of group I, which was exposed to 6.4×10^{-3} joule (6.4×10^{4} ergs) of uv irradiation, was 90 larvae per mouse. Surviving females laid an average of 800 eggs per day; only 3 percent of the eggs were viable. The mean inoculum size of group II, which was exposed to 4.3×10^{-3} joule (4.3×10^{4} ergs) of uv irradiation, was 95 larvae per mouse. Each surviving female laid an average of 200 eggs per day; less than 1 percent of the eggs was viable. The mean yield of adult worms for each group was less than one per mouse, and the yield of males and females was approximately the same.

DISCUSSION

An analysis of variance was indicative that there was no significant difference ($P < 0.05$) in the mean inoculum size among the three

control groups and the two flight experimental groups. The number of adult worms recovered from the two flight experimental groups was not large enough to be considered reliable statistically. The number of adult worms recovered from the ground, vibration, and flight control group mice was similar to data gathered in prelaunch laboratory experiments.

The mean number of eggs recovered from each female worm per day is outlined in figure 7. The recovery from the three control groups and flight experimental group I was between 700 and 875 eggs per female. This egg-production rate is similar to those production rates reported in reference 5. However, the egg-production rate in flight experimental group I was based on a recovery of female worms that were too few in number to be reliable statistically.

The percentage of viable eggs (measured in terms of the percent of eggs hatched) from all groups is shown in figure 8. The percentage of viable eggs obtained from experimental group I and II is too small to be of statistical value. The percentages of viable eggs from the ground and vibration control groups were within 1 percent of each other and are considered reliable as control rates. The percentage of viable eggs from the flight control group fell below the established egg viability control rate. Therefore, a possibility exists that the organisms that were not uv irradiated incurred a reproductive deficiency as a result of passive participation in the 11-day manned space flight mission.

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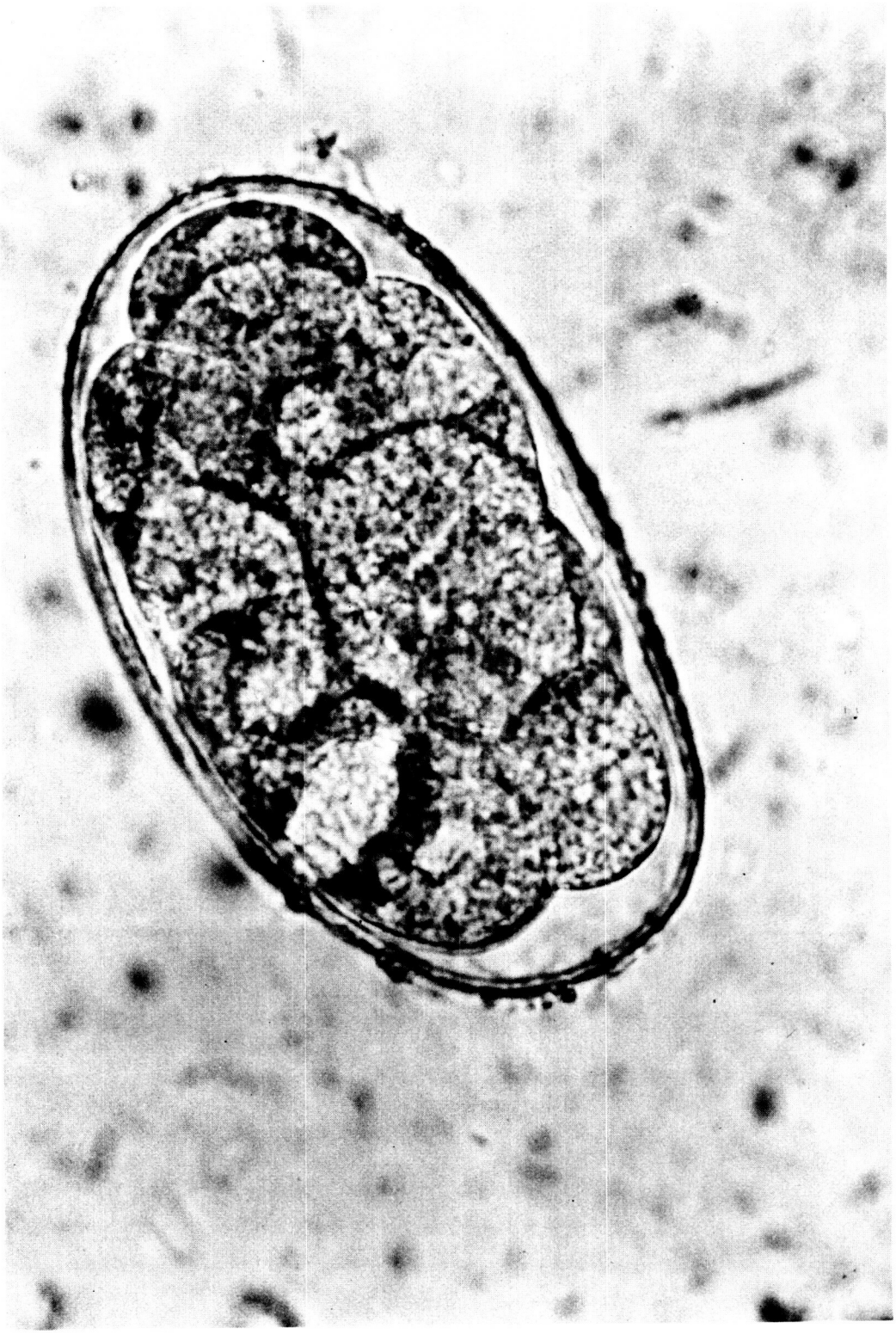

Figure 1.- Egg of Nematospiroides dubius in feces 18 days after inoculation.

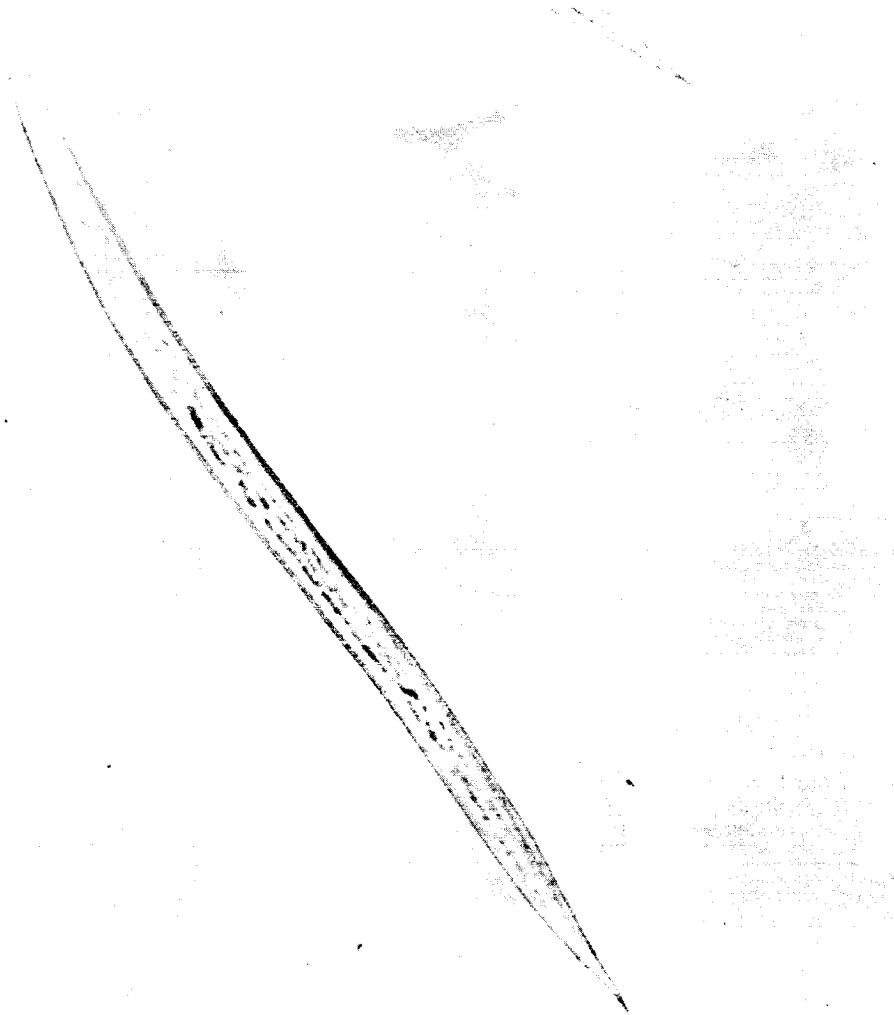

Figure 2.- Infective larva at perimeter of filter paper after 5-day incubation period at 298° K (25° C).

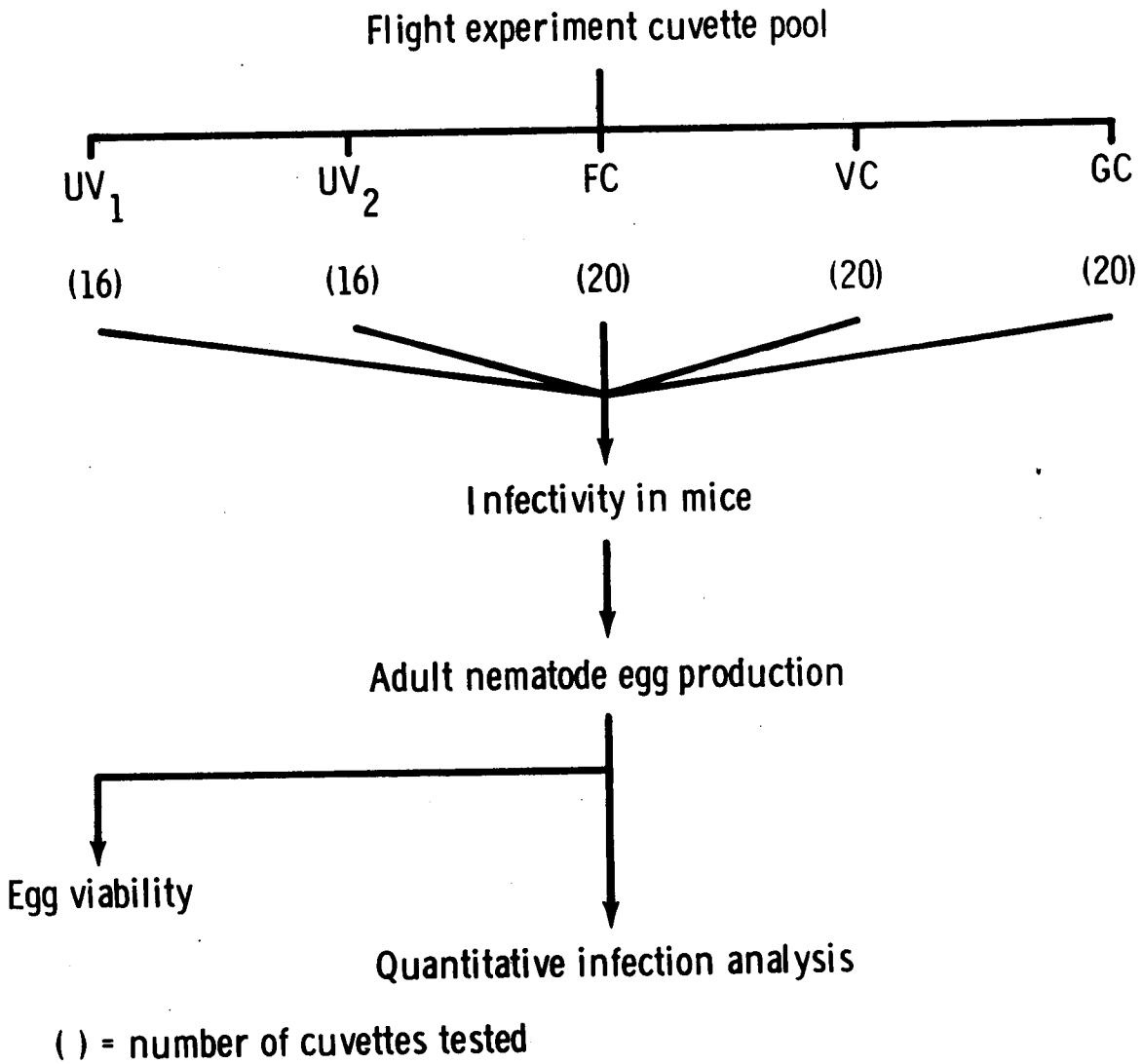

Figure 3.- Nematode test design for Experiment M191.

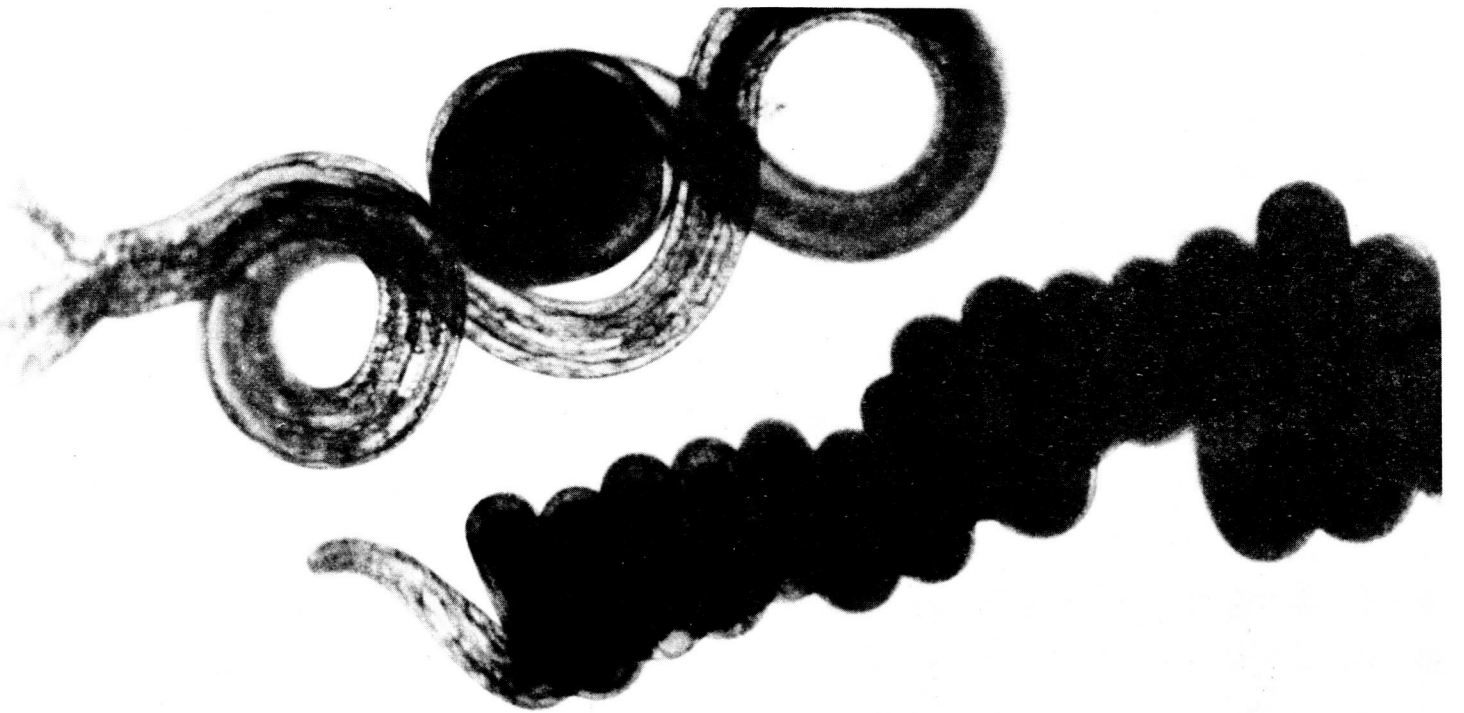

Figure 4.- Adult Nematospiroides dubius from small intestine of mouse.

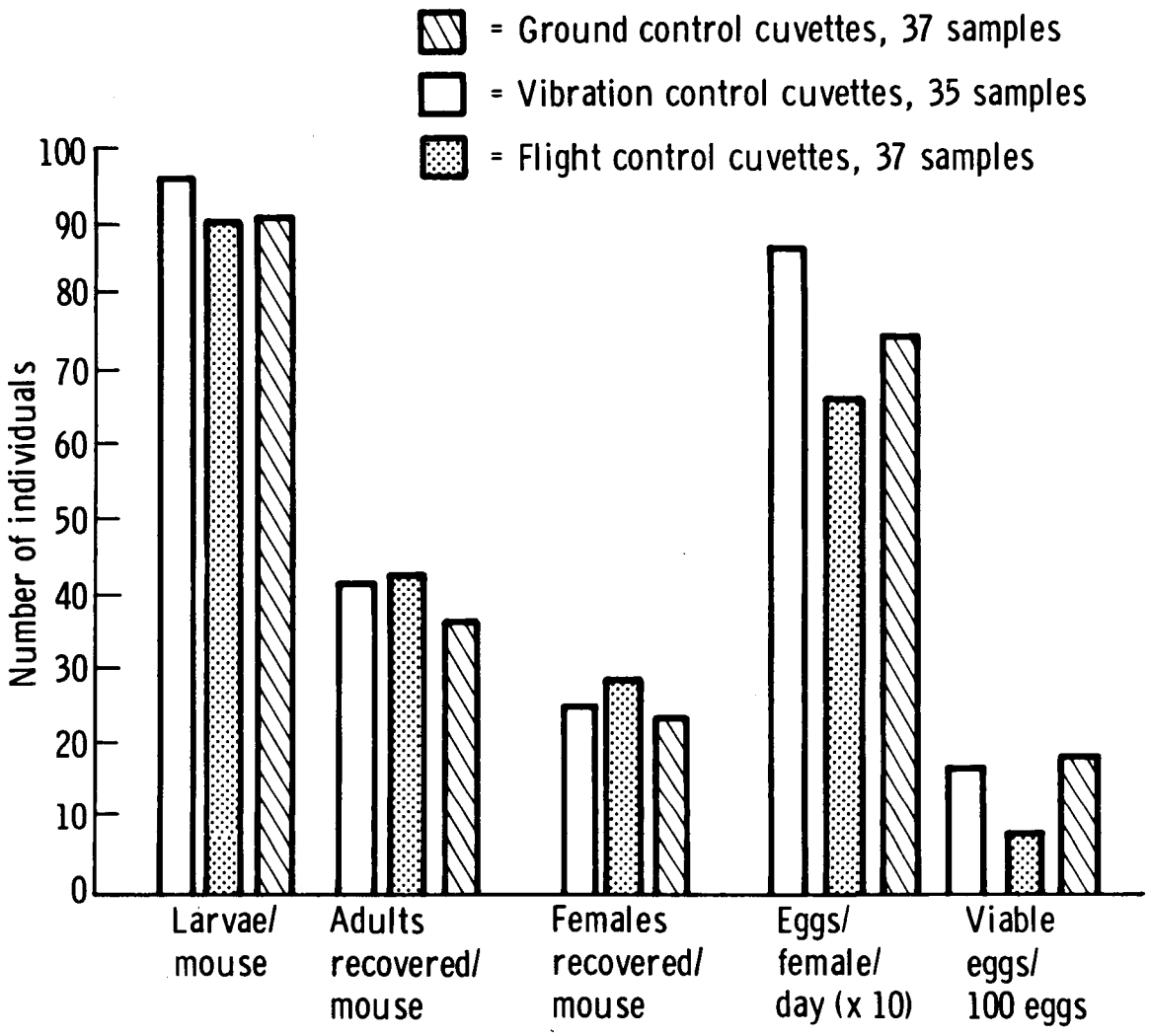

Figure 5.- Results from Nematospiroides dubius in ground control, vibration control, and flight control cuvettes.

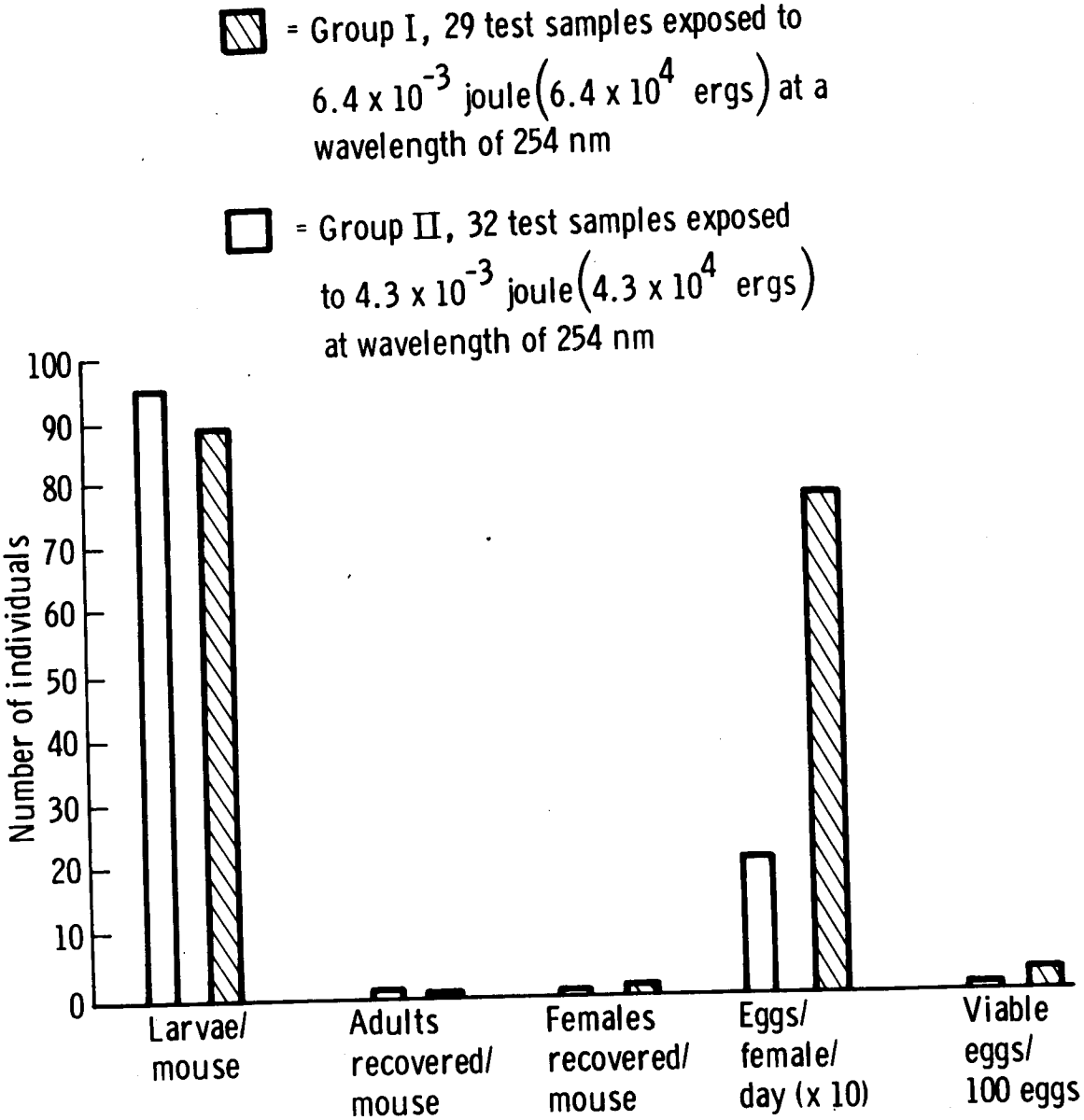

Figure 6.- Summary of flight experimental cuvette data for Nematospiroides dubius.

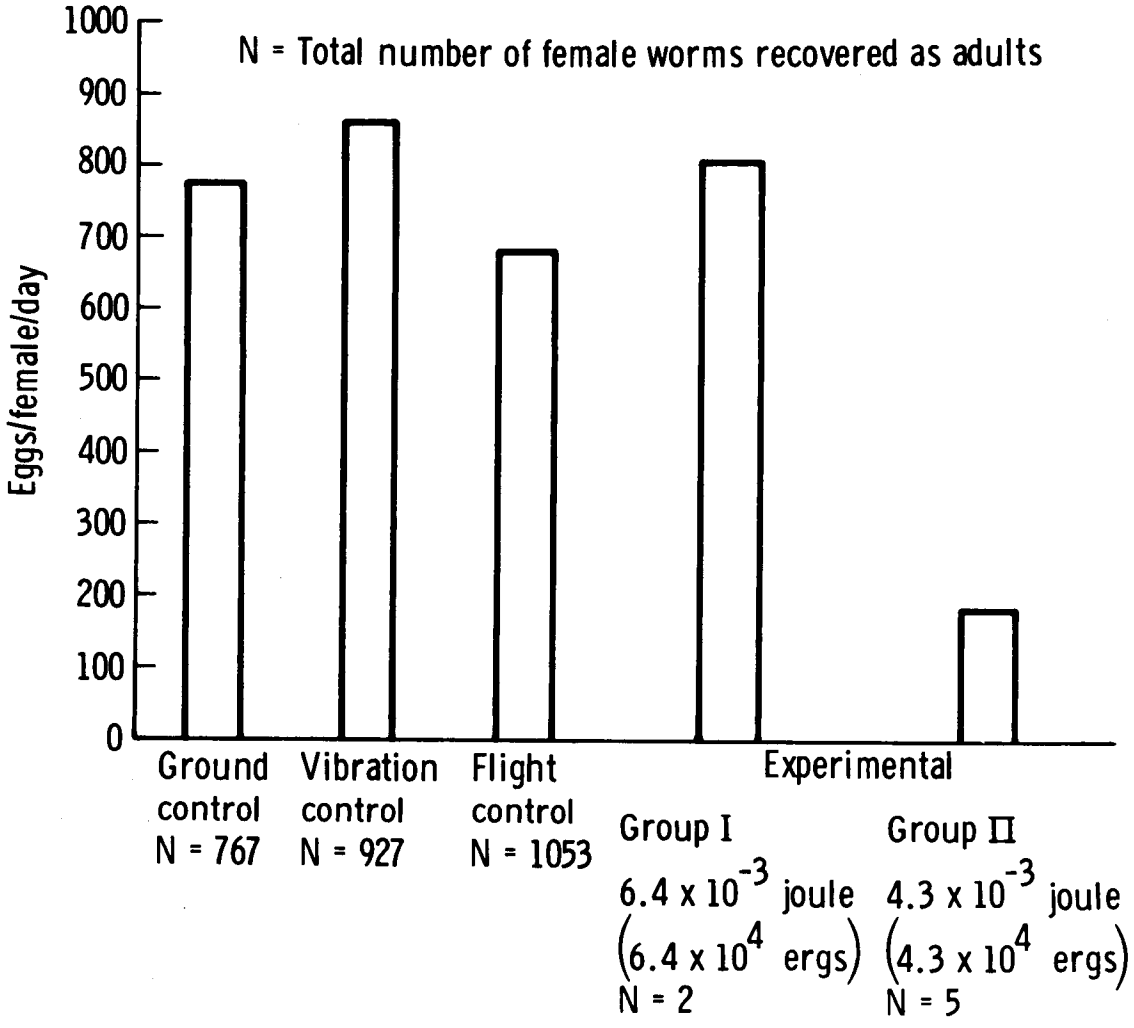

Figure 7.- Egg productivity for Nematospiroides dubius.

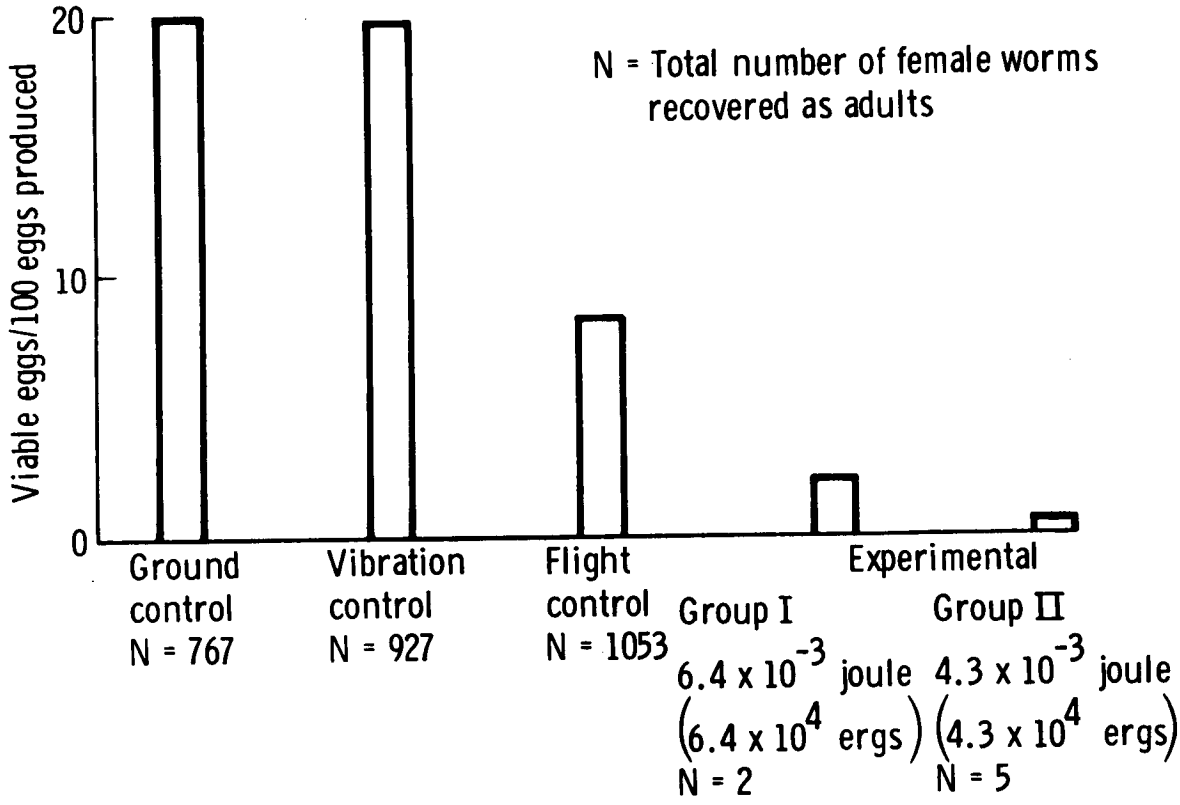

Figure 8.- Viability of Nematospiroides dubius eggs.

EFFECTS OF SPACE ENVIRONMENT ON T-7 BACTERIOPHAGE AND SPORES OF BACILLUS SUBTILIS 168

By John Spizizen* and James E. Isherwood*

ABSTRACT

Spores of Bacillus subtilis and fluid suspensions of the T-7 bacteriophage of Escherichia coli were subjected to space flight environment during the Apollo 16 mission. The results of preliminary studies on returned specimens are discussed.

SUMMARY

Two strains of Bacillus subtilis were exposed to components of the ultraviolet spectrum in space. Both strains possess multiple genetic markers, and one of the strains is defective in the ability to repair ultraviolet damage. The T-7 bacteriophage of Escherichia coli was also exposed to selected wavelengths and energy levels of ultraviolet light in space. Preliminary findings do not reveal anomalies in survival rates. Data are not yet available on detailed genetic analyses.

INTRODUCTION

These studies were designed to investigate the quantitative effects of known irradiations in a space environment on simple microbial systems. Studies in the authors' laboratory have shown that spores of certain repair-defective strains of Bacillus subtilis are sensitive to the lethal effects of ultraviolet (uv) irradiations. In addition, the authors have found that the mutagenic effects of irradiations can be detected readily by employing morphological and sporulation-defective phenotypes arising from mutations in numerous genes. The stability of irradiation-induced lesions in spores provides an ideal microbial monitoring system for

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quantitating the effects of irradiations and other environmental factors encountered in space. In addition to the spore system, the T-7 bacteriophage of Escherichia coli was used to detect the lethal effects of uv irradiations in space.

MATERIALS AND METHODS

The two strains of Bacillus subtilis 168 used in these studies were designated HA 101 and HA 101 (59)F. These strains were obtained from Dr. Kenneth Gass of the University of Chicago (ref. 1). Both HA 101 and HA 101 (59)F require histidine, leucine, and methionine for growth. Strain HA 101 (59)F is sensitive to methyl methane sulfonate (MMS), ethyl methane sulfonate (EMS), and uv irradiation at a wavelength of 254 nanometers. It is also deficient in deoxyribonucleic acid (DNA) polymerase I (ref. 1).

These strains produce characteristic rough colonies on sporulation AK agar (Baltimore Biological Laboratories), where sporulation-defective mutant colonies, induced by mutagenic agents, appeared as morphological variants (fig. 1). Other morphological mutants that can sporulate also occurred after mutagenesis. Each of the variant colonies was restreaked on minimal agar containing two of the required three amino acids and on minimal agar containing all three amino acids. Growth on minimal agar containing the three required amino acids and no growth in the absence of any one of the three amino acids was indicative that the clone was derived from the test strains. This evaluation was required in order to eliminate contaminants. Defective spore production was indicated by the absence of spores on AK agar after 72 hours, as determined by phase-contrast microscopy.

Spores of these strains were grown on tryptose blood agar base (TBAB) or on sporulation AK agar (Baltimore Biological Laboratory) for 48 hours at 310° K (37° C) and were suspended in distilled water at a concentration of 10^{10} spores/cm^3 (10^{10} spores/ml). After treatment with lysozyme, the spore suspension was centrifuged and suspended in 15-percent Renografin-76. A gradient was prepared with 20 cm^3 (20 milliliters) of 70-percent Renografin-76 followed by 20 cm^3 (20 milliliters) of 30-percent Renografin-76. A spore suspension of 6 cm^3 (6 milliliters) in 15-percent Renografin-76 was layered on the gradient. After centrifugation for 40 minutes at a speed of 16 000 rpm (30 900 × g), the pellet, containing refractile spores, was suspended in cold, sterile, distilled water and was washed five times with cold water. Spore suspensions were stored in water at 278° K (5° C) and were tested for uv sensitivity after 1 week, when they were optimally disaggregated.

In ground-based experiments, 1.8-cm^3 (1.8 milliliter) aliquots of spore suspensions, not exceeding 10^8 spores/cm^3 (10^8 spores/ml), were irradiated in small Petri dishes. Ground control spore monolayers also were prepared on 0.45-micrometer Millipore membrane filters by filtration of aqueous suspensions; ultimately, approximately 10^8 spores were attached to a 25-millimeter-diameter filter. The monolayers were subsequently dried at 293° K (20° C) for 1 hour. Spores were recovered after irradiation by suspending the filters in a 0.025-percent solution of Tween 80 and by sonicating for 15 minutes.

For flight studies, purified spores were diluted to 5×10^7 spores/cm^3 (5×10^7 spores/ml) in distilled water. Wet (type A) cuvettes contained 2.5×10^6 spores in 0.05 cm^3 (50 microliters) of fluid. Dry (types B and C) cuvettes contained spore monolayers on Millipore filters prepared as follows. First, 3.8 cm^3 (3.8 milliliters) of the diluted spore suspension were filtered on a 47-millimeter Millipore type HA filter (0.45-micrometer pore diameter, 960-mm^2 area). The filters were dried on filter paper in Petri dishes for approximately 1 hour. Ten to 12 chips (25 mm^2 each) were punched from each filter under sterile conditions. Thus, the number of spores per chip was

$$\frac{3.85 \times 5 \times 10^7 \times 25\ mm^2}{960\ mm^2} = 5 \times 10^6 \qquad (1)$$

Spores from type A cuvettes were recovered by prior sonication of the cuvettes for 1 minute in a bath of 70-percent ethanol. After drying the outside of the cuvette, the contents were removed with a 1-cm^3 (1 milliliter) syringe, and the inside was rinsed five times with 1.95 cm^3 (1.95 milliliters) of distilled water containing 0.025 percent Tween 80. The pooled contents were stored at 278° K (5° C) and were assayed for viable counts and morphological mutants on AK agar plates. Spores from types B and C cuvettes were recovered by transferring chips from cuvettes to empty vials. The cuvettes and inside cuvette windows were rinsed with 2.0 cm^3 (2.0 milliliters) of a 0.025-percent Tween 80 solution and were added to the vials containing chips. The vials were sonicated for 15 minutes to remove spores from the filters. Viable-count assays were made on AK agar plates.

The T-7 bacteriophage used in this study was supplied originally by Dr. S. E. Luria. The host, Escherichia coli B, was grown at 310° K (37° C) in a medium containing the following chemicals.

Disodium phosphate, NA_2HPO_4, g/m^3 (g/liter)	0.0110 (11.0)
Potassium hydrogen phosphate, KH_2PO_4, g/m^3 (g/liter)	0.0045 (4.5)
Ammonium chloride, NH_4Cl, g/m^3 (g/liter) . . .	0.0030 (3.0)
Potassium hydroxide, KOH, ...	To pH = 7
Glucose, $C_6H_{12}O_6$, percent	0.5
Magnesium sulfate, $MgSO_4$, ...	To mM = 1

When the cell density became approximately 1.4×10^9 cells/cm^3 (1.4×10^9 cells/ml), the following components were added: $MgSO_4$, 0.2 millimolar; calcium chloride ($CaCl_2$), 0.2 millimolar; and T-7 phage at a multiplicity of 1 phage/10 host cells. Lysis occurred after 2 hours, at which time Tris hydrochloric acid (Tris HCl), 30 millimolar; sodium chloride (NaCl), 1 molar; and 61×10^{-3} kg/cm^3 (61 g/liter) of Carbowax (polyethylene glycol 20 000) were added. The lysate was held at 277° K (4° C) for 24 hours. The phage was centrifuged at a speed of 8000 rpm for 30 minutes and was purified in a cesium chloride density gradient at a speed of 22 000 rpm for 2.5 hours. The purified phage was stored in 20 millimolar Tris (pH 7.5), 0.5 molar NaCl, and 20 $\mu g/cm^3$ (20 μg/ml) gelatin solution. Concentrated stocks were diluted to 1×10^8 plaque-forming units/cm^3 (1×10^8 plaque-forming units/ml) for ground-based and flight experiments. For flight experiments, each cuvette contained 0.05 cm^3 (0.05 milliliters), or 5×10^6 phage units. Phage assays were performed using Escherichia coli B as indicator host on TBAB agar.

In ground-based experiments, uv irradiation was generated using either a germicidal lamp or a quartz/mercury-vapor lamp. The germicidal lamp emission was measured with a uv meter; most emissions were at a wavelength of 254 nanometers. Filters were used with the quartz/mercury-vapor lamp to provide maximal emissions at wavelengths of 254, 280, and 300 nanometers.

RESULTS

Ground-Based Experiments

Extensive ground-based studies have been performed that provide reproducible dose-survival data for the two spore types and T-7 coliphage. The survival (N/N_0, where N = number of survivors and N_0 = original number) data of spores (in aqueous suspension) of strains HA 101 and HA 101 (59)F at different energy levels of irradiation from a germicidal lamp are shown in figure 2. The numbers adjacent to each point are the percentage of morphological mutants. Evidently, the HA 101 (59)F strain is more sensitive to the 254-nanometer wavelength and is less mutable, particularly at higher energy levels. Strain HA 101 appears to be more mutable at higher energy levels. Survival rates of the spores dried on Millipore surfaces as a function of different energy levels, using the quartz/mercury-vapor lamp and filter transmitting mainly a 254-nanometer wavelength, are plotted in figure 3. Evidently, spores exposed in this manner are more sensitive to the lethal effects of 254-nanometer irradiation. The HA 101 (59)F strain is considerably more sensitive and less mutable than is the HA 101 strain. A mutation frequency decline as a function of increasing energy levels appears to be characteristic of the HA 101 (59)F strain but not of the HA 101 strain. Data on the lethal and mutagenic effects of irradiation at 280 nanometers wavelength on spores that were dried on Millipore filters are plotted in figure 4. It is evident that the HA 101 (59)F strain is more sensitive. Mutation frequencies resulting from a wavelength of 280 nanometers are similar to survival levels resulting from a wavelength of 254 nanometers. However, the lethal effects of irradiation at a wavelength of 280 nanometers appear to be greater than for the same energy levels of a 254-nanometer wavelength.

The morphological mutants were restreaked on minimal agar that contained combinations of the required amino acids, and the mutants were scored for sporulation by phase-contrast microscopy. Data from ground-based experiments with 254- and 280-nanometer wavelengths at different energy levels using a quartz/mercury-vapor lamp with appropriate filters are contained in table I. The percentage of mutants relative to survivors is indicated at each energy level. The mutation frequencies for morphological and asporogenous phenotypes are maximum at low dose levels for the HA 101 (59)F strain and diminish at higher doses of radiation. However, for the HA 101 strain, the mutation frequencies are higher with higher energies at both wavelengths. Mutation frequencies for selective markers (prototrophy, resistance to azide, erythromycin, and 5-methyl tryptophan) currently are being investigated but are of considerably lower magnitude.

The inactivation of T-7 phage suspensions by different energy levels of the 254-nanometer wavelength is represented in figure 5. The biphasic nature of the inactivation curve is indicative of repair at low doses by the E. coli B host. This possibility is being examined by the use of excision repair-defective mutants (uvr^-) of E. coli B. Nevertheless, the characteristics of the response with the host used are valuable for comparison with space flight studies.

Flight Data (Preliminary)

Controls.- An extensive study of spores and phage not exposed to light (controls) was made to evaluate stabilities, recoveries, effects of vibration, and other factors encountered in flight. The viable count data (N_0) for the two B. subtilis strains and T-2 phage are summarized in table II. Vial samples were held in the authors' laboratory at temperatures similar to the flight hardware. Ground controls were held in flight cuvettes and accompanied the flight hardware at all times except during flight. Vibration controls were subjected to simulated launch vibrations in flight cuvettes at the NASA Lyndon B. Johnson Space Center (JSC), and flight controls were placed in the flight experiment package but were not exposed to light. Because all values were within the errors of measurements, the data are indicative that the N_0 values of these controls were not altered by flight conditions. Analysis of morphological and sporulation mutants from the control samples was indicative of low frequencies, and no effect of space flight conditions in the absence of light could be detected (table III).

Type A cuvettes (aqueous samples).- Survival ratios (N/N_0) and the frequencies of morphological and sporulation mutants varied considerably in the type A cuvettes exposed to 254- and 280-nanometer-wavelength irradiations. This variation may be caused by bubbles, leakage, and other technical problems related to recovery of the material. The data obtained with spores of strains HA 101 and HA 101(59)F at the two wavelengths are summarized in table IV. From these data, considerably lower inactivation was obtained than would have been predicted from ground-based experiments, assuming that the energy levels were determined correctly. Also, the frequencies of mutations are considerably lower than the expected numbers. In the case of the T-7 phage, however, the inactivation was considerably higher than that obtained with ground experiments (table V), although the shapes of the dose-response curves are similar (fig. 5).

Type B cuvettes (dry, unvented samples).- More consistent results were obtained with spores of the two strains in type B cuvettes exposed to irradiation at wavelengths of 254 and 280 nanometers. Higher levels

of inactivation were obtained at similar energy levels compared with ground-based studies, except with low doses. Generally, mutation frequencies were similar (tables VI and VII). A reevaluation of the energy data may resolve some of the discrepancies.

The effect of full light is of some interest. Although inactivation was not as high for both strains as was expected, the frequencies of mutation were elevated in the HA 101 (59)F strain for similar doses in ground-based experiments.

Type C cuvettes (dry, vented samples).- Although a limited number of energy levels were used for the type C cuvette series, both lethal and mutagenic effects of irradiation at 254- and 280-nanometer wavelengths were in general agreement with those effects observed for the type B cuvettes (table VIII). At one energy level, 2.7×10^{-4} joule (2.7×10^{3} ergs) of 254 nanometer-wavelength irradiation, an unusually high mutation frequency was obtained with the HA 101 strain. Unfortunately, the energy levels selected were not sufficiently high to obtain more definitive data with this system.

CONCLUSIONS

Although the results are preliminary, a number of conclusions can be stated.

1. Spores of Bacillus subtilis and T-7 bacteriophage are satisfactory for flight studies because they are stable and recoverable in the absence of lethal irradiations.

2. Aqueous suspensions were less satisfactory than spore monolayers on Millipore filters as far as reproducible results were concerned. Presumably, this condition was caused by technical problems of filling and recovery.

3. In the absence of solar irradiation, there was no significant effect of flight conditions on survival and mutation frequency of spores of the two strains used.

4. Generally, the lethal effects of irradiation at 254- and 280-nanometer wavelengths were greater for spores exposed on Millipore filters. The repair-defective strain was more sensitive at both wavelengths. Mutation frequencies were similar to mutation frequencies from ground-based tests. However, an unusually high mutation frequency was obtained with the HA 101 strain in vented cuvettes at one energy level

of 254-nanometer-wavelength irradiation. Also, elevation of mutation frequency was observed in the repair-defective strain when this strain was exposed to full light during the flight experiment.

5. The lethal effect of 254-nanometer-wavelength irradiation was considerably higher for T-7 bacteriophage at all energy levels compared with ground-based experiments. The characteristic shape of the dose-response curve was similar to the curve of the ground experimental data. Because of the discrepancies observed, the data on energy levels need to be reassessed.

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TABLE I.- MUTANT FREQUENCIES IN GROUND-BASED STUDIES OF

BACILLUS SUBTILIS SPORES, IRRADIATED ON MILLIPORE FILTERS

Wavelength = 254 nm			Wavelength = 280 nm		
Energy, joule/cm^2 × 10^{-5} (ergs/cm^2 × 10^2)	Morphological mutant, percent	SP$^-$ mutant, percent	Energy, joule/cm^2 × 10^{-5} (ergs/cm^2 × 10^2)	Morphological mutant, percent	SP$^-$ mutant, percent
HA 101 (59)F strain					
0.00	0.08	0.08	0.0	0.00	0.00
41.50	.97	.29	14.4	.45	.06
83.00	4.52	1.23	24.0	.58	.43
124.50	4.45	1.27	28.8	2.36	.81
145.25	6.18	2.53	38.4	4.59	1.71
166.00	2.44	.72	48.0	4.64	1.58
249.00	2.31	.93	57.6	4.46	1.24
290.50	2.65	.61	72.0	3.19	1.75
332.00	2.23	.56	96.0	3.26	1.16
--	--	--	120.0	2.72	1.36
--	--	--	144.0	2.90	.53
HA 101 strain					
0.00	0.30	0.30	0.0	0.00	0.00
41.50	.58	.19	24.0	.42	.25
103.75	1.52	1.08	38.4	1.10	.40
207.50	2.14	1.03	57.6	1.36	.63
311.25	7.60	3.80	72.0	3.51	.99
332.00	5.70	2.51	96.0	3.93	1.47
518.75	7.43	5.09	120.0	5.87	2.63
664.00	5.31	2.46	144.0	6.99	2.14
830.00	6.81	4.25	192.0	8.30	3.69
1328.00	7.87	6.30	--	--	--

TABLE II.- VIABILITY OF CONTROLS

$\left[N_0 = \text{survivors}/10^6\ \text{cm}^3\right]$

Location	*Bacillus subtilis* HA 101 (59)F strain, cuvette type —			*Bacillus subtilis* HA 101 strain, cuvette type —			T-7 phage, cuvette type A (a)
	A (a)	B (b)	C (c)	A (a)	B (b)	C (c)	
Vial	4.30	5.46	6.25	3.77	4.66	4.61	--
Ground	3.78	5.52	5.43	3.17	5.02	4.72	5.61
Vibration	4.12	6.10	5.34	2.95	4.92	4.86	5.45
Flight	3.69	6.18	6.17	3.30	5.14	4.71	4.45

[a]Aqueous samples.

[b]Nonvented, spores on filters.

[c]Vented, spores on filters.

TABLE III.- MUTANT FREQUENCIES OF BACILLUS SUBTILIS SPORES IN GROUND AND FLIGHT CONTROLS

Cuvette type	Location	Morphological mutants, percent	SP^- mutants, percent
HA 101 (59)F strain			
[a]A	Vial	0.08	0.05
	Ground	.17	.11
	Flight	.08	.05
	Vibration	.09	.06
[b]B	Vial	0.06	0.07
	Ground	.15	.08
	Flight	.01	.01
	Vibration	.03	.01
[c]C	Vial	0.04	0
	Ground	.14	0
	Flight	.19	.11
	Vibration	.07	0
HA 101 strain			
A	Vial	0.03	0
	Ground	.02	0
	Flight	.03	0
	Vibration	.02	0
B	Vial	0.05	--
	Ground	.07	0.04
	Flight	.07	.03
	Vibration	.05	.03
C	Vial	0.26	--
	Ground	.04	--
	Flight	.06	--
	Vibration	.02	--

[a]Aqueous samples. [c]Vented, spores on filters.

[b]Nonvented, spores on filters.

TABLE IV.- FLIGHT RESULTS OF BACILLUS SUBTILIS SPORES RECOVERED FROM WET (TYPE A) CUVETTES

Strain	Energy		Average N/N_0	Average morphological mutants, percent	Average SP^- mutants, percent
	Joule/25mm^2/ 10 min	Ergs/25mm^2/ 10 min			
Wavelength = 254 nm					
HA 101	9.0×10^{-4}	9.0×10^{3}	0.40	2.24	1.76
	2.0×10^{-3}	2.0×10^{4}	.13	1.51	1.22
	3.3×10^{-3}	3.3×10^{4}	.051	.51	.43
HA 101 (59)F	5.4×10^{-4}	5.4×10^{3}	0.060	1.28	0.30
	2.1×10^{-3}	2.1×10^{4}	.24	.25	.19
	8.7×10^{-3}	8.7×10^{4}	.39	.39	.11
Wavelength = 280 nm					
HA 101	2.3×10^{-3}	2.3×10^{4}	0.77	0.34	0.08
	4.2×10^{-3}	4.2×10^{4}	.41	1.60	.87
	6.5×10^{-3}	6.5×10^{4}	.12	2.29	1.35
HA 101 (59)F	8.1×10^{-4}	8.1×10^{3}	0.65	0.77	0.80
	1.2×10^{-3}	1.2×10^{4}	.28	.75	.39
	3.1×10^{-3}	3.1×10^{4}	.33	.21	.21

TABLE V.- FLIGHT AND GROUND-BASED RESULTS OF T-7 PHAGE RECOVERED FROM WET (TYPE A) CUVETTES IRRADIATED AT A WAVELENGTH OF 254 nm

Energy		Experimental N/N_0 (flight)	Ground N/N_0 (a)
Joule/25mm^2/ 10 min	Ergs/25mm^2/ 10 min		
1.4×10^{-5}	1.4×10^{2}	0.36	1.00
4.9×10^{-5}	4.9×10^{2}	.14	.64
1.2×10^{-4}	1.2×10^{3}	.036	.45
2.6×10^{-4}	2.6×10^{3}	.014	.30
5.4×10^{-4}	5.4×10^{3}	.001 4	.16
1.3×10^{-3}	1.3×10^{4}	.000 01	.01

[a]Ultraviolet source, germicidal lamp.

TABLE VI.- FLIGHT RESULTS OF BACILLUS SUBTILIS (HA 101) SPORES RECOVERED FROM DRY (TYPE B) CUVETTES

Energy		Ground N/N_0 (a)	Average N/N_0 (b)	Morphological mutants, percent	SP^- mutants, percent
Joule/25mm^2/ 10 min	Ergs/25mm^2/ 10 min				
Wavelength = 254 nm					
1.7×10^{-4}	1.7×10^{3}	0.91	1.27	0.36	0.13
5.4×10^{-4}	5.4×10^{3}	.80	.60	4.34	3.37
1.4×10^{-3}	1.4×10^{4}	.41	.024	2.93	2.14
2.1×10^{-3}	2.1×10^{4}	.20	.009	3.35	2.29
Wavelength = 280 nm					
4.1×10^{-4}	4.1×10^{3}	0.55	1.11	0.17	0
8.1×10^{-4}	8.1×10^{3}	.25	.36	.83	.40
2.3×10^{-3}	2.3×10^{4}	.03	.10	5.60	3.13
4.2×10^{-3}	4.2×10^{4}	<.01	.095	6.53	3.42
Full, unfiltered light					
1.4×10^{1}	1.4×10^{8}	--	0.0045	2.83	1.60

[a] Quartz/mercury-vapor lamp with filter.

[b] Experimental N_0 (ground control) = 4.97×10^{6}.

TABLE VII.- FLIGHT RESULTS OF BACILLUS SUBTILIS HA 101 (59)F SPORES RECOVERED FROM DRY (TYPE B) CUVETTES

Energy		Ground N/N_0 (a)	Average N/N_0 (b)	Morphological mutants, percent	SP^- mutants, percent
Joule/25mm^2/ 10 min	Ergs/25mm^2/ 10 min				
Wavelength = 254 nm					
8.2×10^{-5}	8.2×10^{2}	>0.95	0.57	1.30	0.64
2.6×10^{-4}	2.6×10^{3}	.74	.33	3.22	.15
5.4×10^{-4}	5.4×10^{3}	.33	.001 0	1.78	2.29
8.7×10^{-3}	8.7×10^{4}	--	.018	1.02	.84
Wavelength = 280 nm					
1.1×10^{-4}	1.1×10^{3}	0.97	0.48	0.27	1.03
4.1×10^{-4}	4.1×10^{3}	.21	.16	2.06	3.01
8.1×10^{-4}	8.1×10^{3}	.013	.59	3.90	.55
2.3×10^{-3}	2.3×10^{4}	< .001	.004 7	1.38	.97
Full, unfiltered light					
1.4×10^{1}	1.4×10^{8}	--	0.000 23	2.57	1.64

[a]Ultraviolet source, quartz/mercury-vapor lamp with filter.

[b]Experimental N_0 (ground control) = 5.91×10^{6}.

TABLE VIII.- FLIGHT RESULTS OF BACILLUS SUBTILIS SPORES RECOVERED FROM DRY (TYPE C) CUVETTES (VENTED)

Wavelength, nm	Energy, joule/25mm^2/10 min × 10^{-4} (ergs/25mm^2/10 min × 10^3)	N/N_0	Morphological mutants, percent
HA 101 strain			
254	1.3	0.89	0.26
254	2.7	.62	3.10
280	1.6	1.05	.17
280	4.1	1.09	.36
HA 101 (59)F strain			
254	1.3	0.29	1.13
254	2.7	.035	1.19
280	1.6	.82	.36
280	4.1	.15	2.34

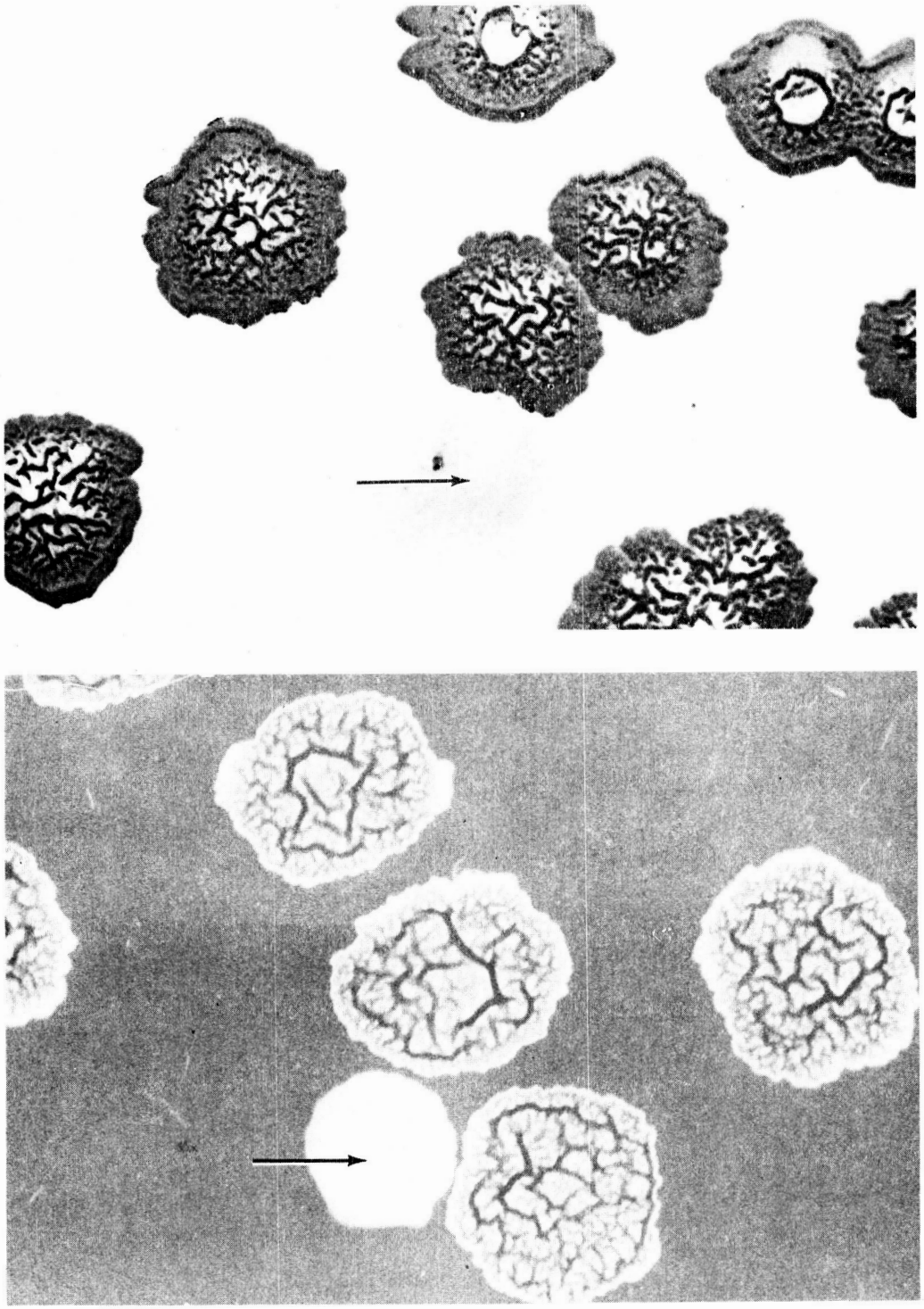

Figure 1.- Appearance of morphological mutants on AK sporulation agar.

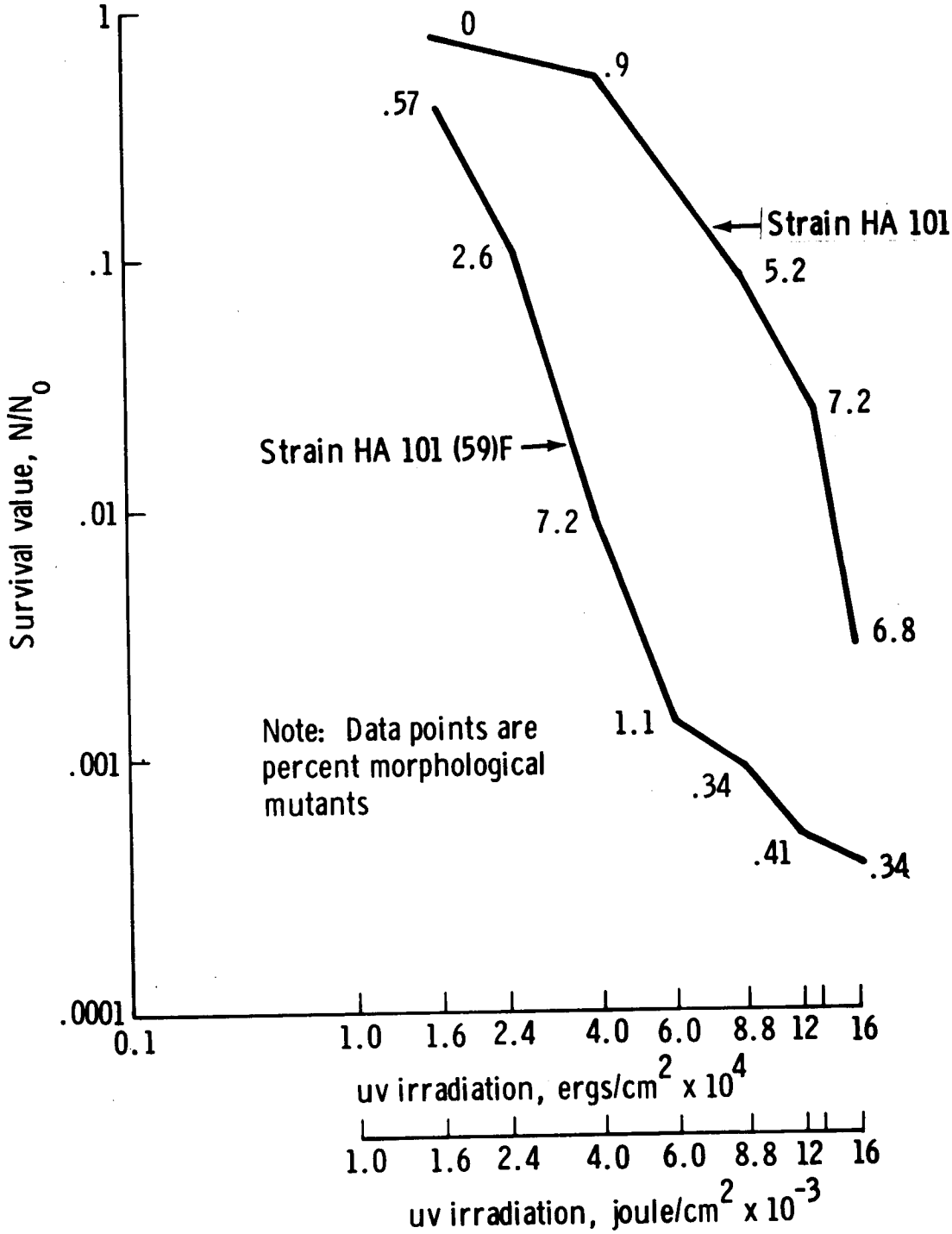

Figure 2.- Survival and mutation frequency of B. subtilis spores (uv irradiation by germicidal lamp with major emission of 254 nm).

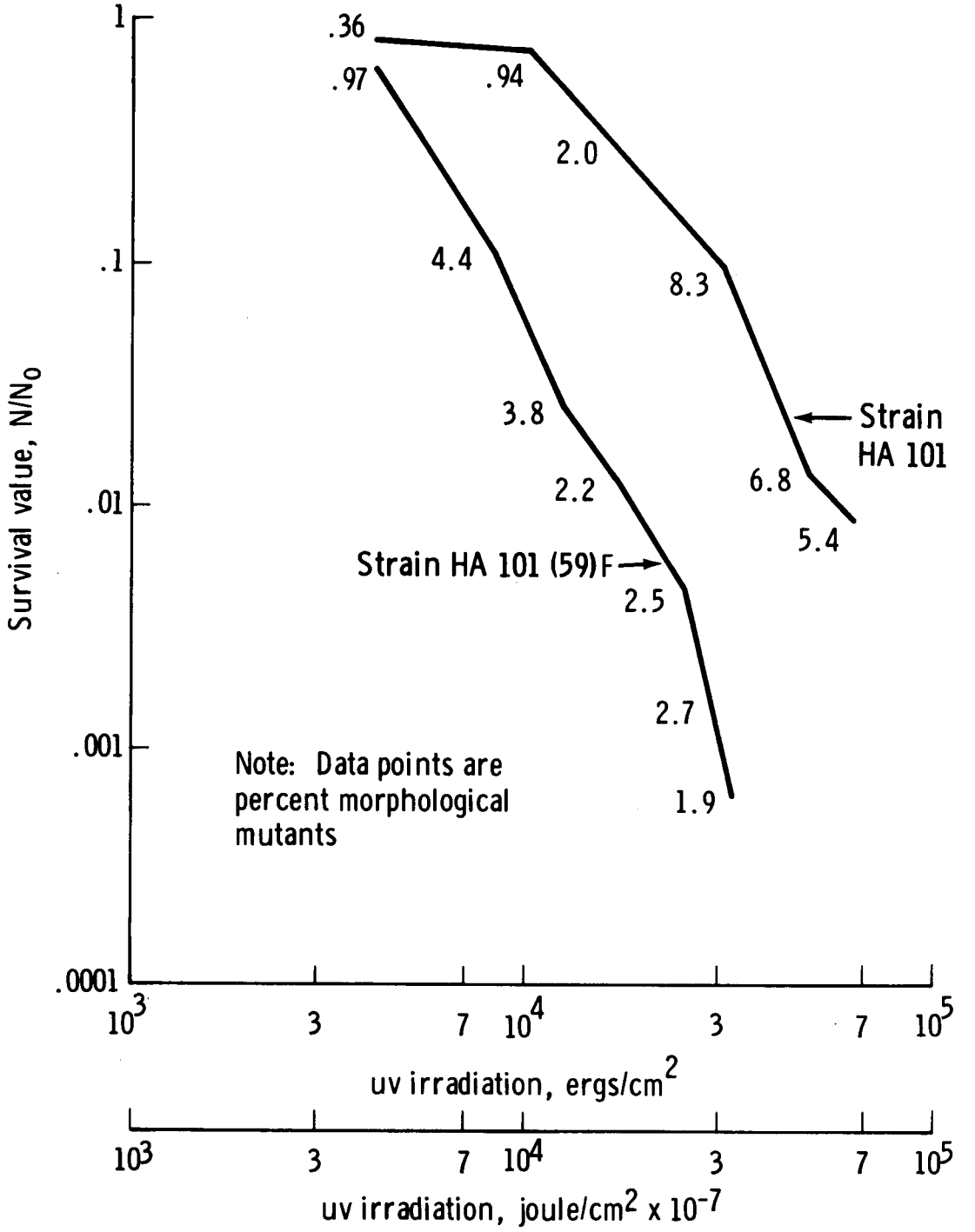

Figure 3.- Survival and mutation frequency of B. subtilis spores (uv irradiation by quartz/mercury-vapor lamp with major emission at 254 nm).

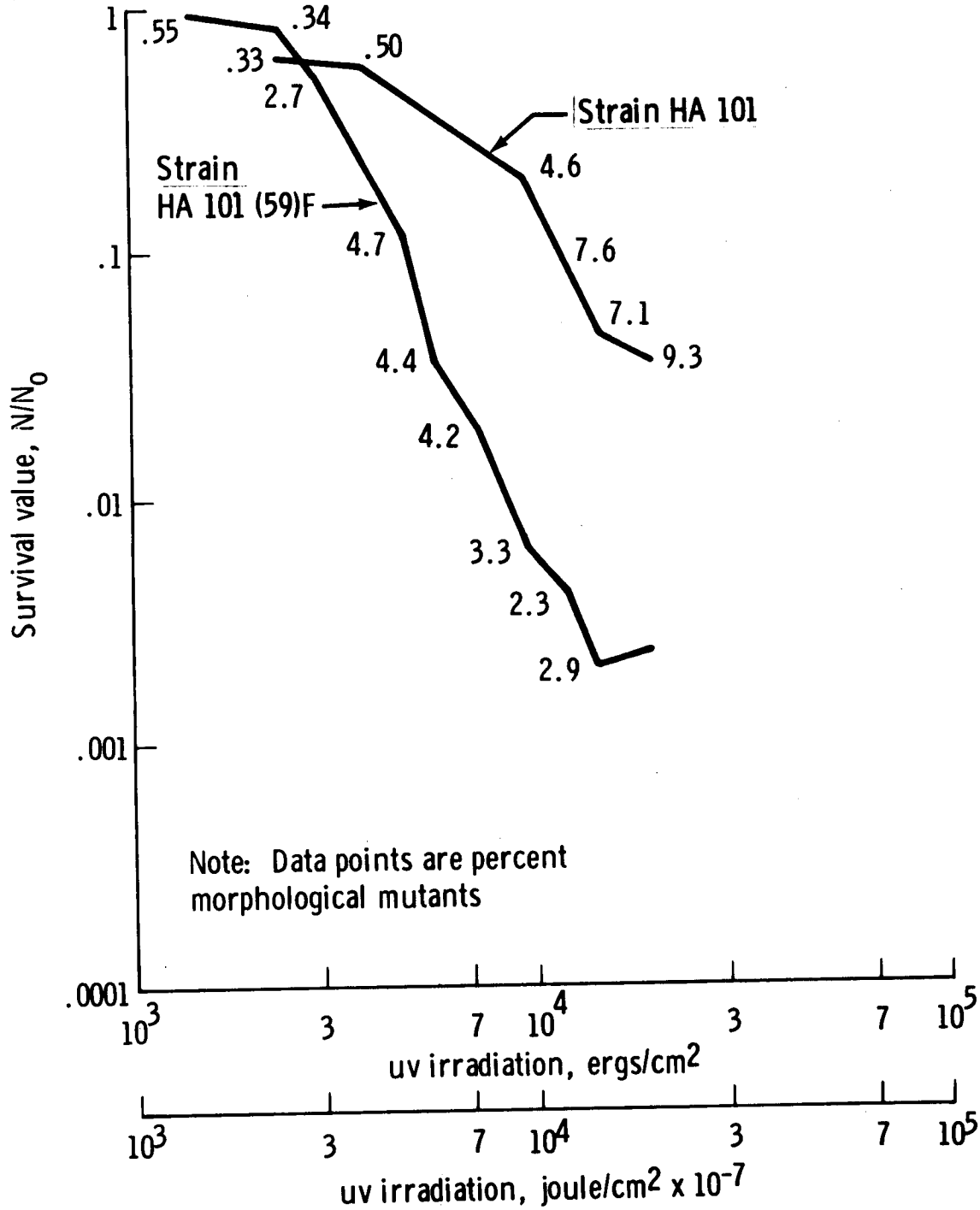

Figure 4.- Survival and mutation frequency of B. subtilis spores (uv irradiation by quartz/mercury-vapor lamp with major emission at 280 nm).

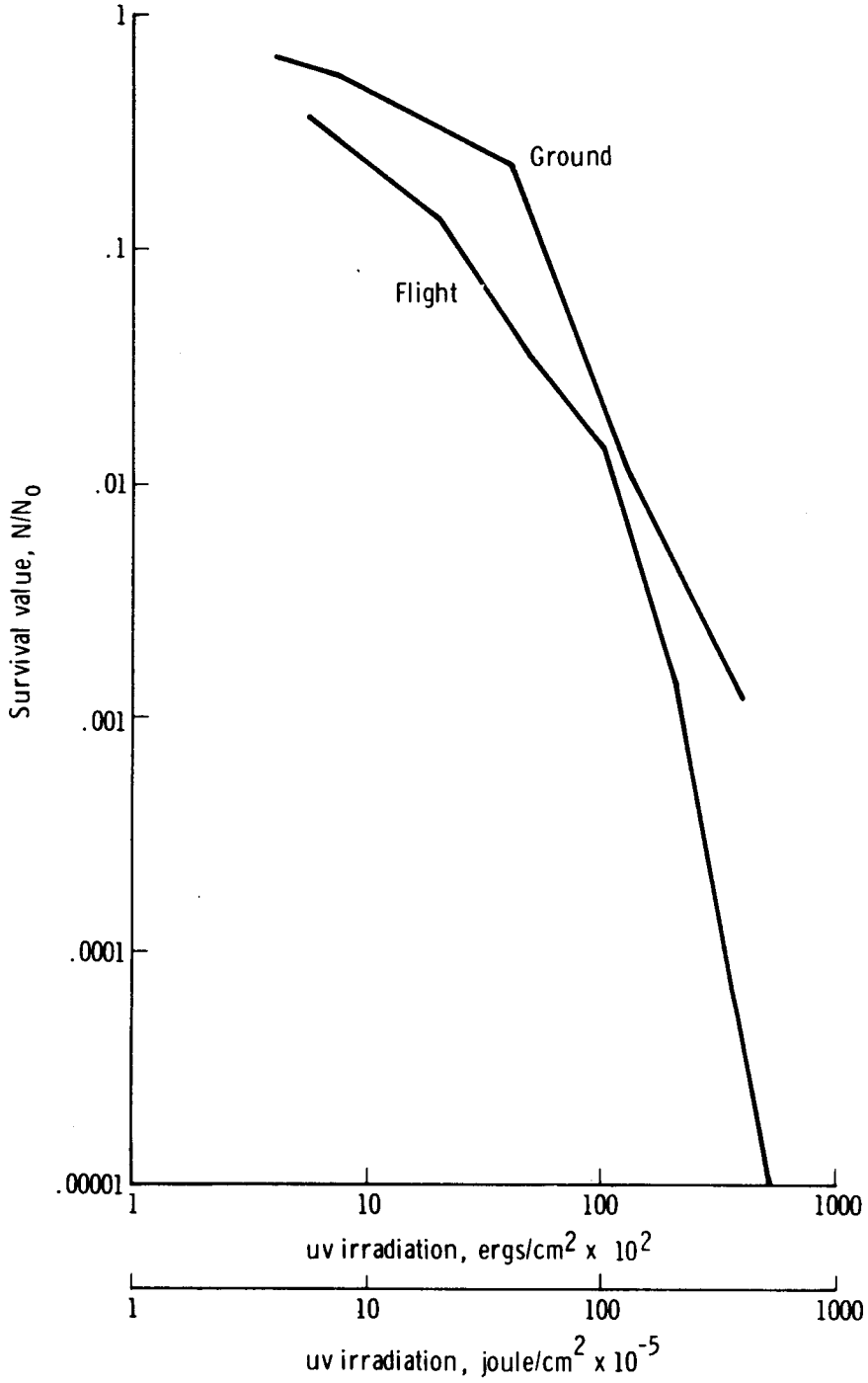

Figure 5.- Survival of E. coli T-7 Phage (uv irradiation by germicidal lamp with major emission at 254 nm).

EFFECTS OF SPACE VACUUM AND SOLAR ULTRAVIOLET IRRADIATION (254 NANOMETERS) ON THE COLONY-FORMING ABILITY OF *BACILLUS SUBTILIS* SPORES

By Horst Bücker,* Gerda Horneck,* and Helga Wollenhaupt*

ABSTRACT

Bacillus subtilis spores are highly resistant to harsh environments. Therefore, in the Apollo 16 Microbial Response to Space Environment Experiment (M191), these spores were exposed to space vacuum or solar ultraviolet irradiation, or both, to estimate the chance of survival for terrestrial organisms in space. The survival of the spores was determined in terms of colony-forming ability. Comparison of the flight results with results of simulation experiments on earth applying high vacuum or ultraviolet irradiation, or both, revealed no remarkable difference. Simultaneous exposure to both these space factors resulted in a synergistic effect (that is, an ultraviolet supersensitivity). Therefore, the chance of survival in space is assumed to depend on the degree of protection against solar ultraviolet irradiation.

SUMMARY

The experiment on *Bacillus subtilis* spores is part of the Apollo 16 Microbial Response to Space Environment Experiment (M191). The spores were exposed to space vacuum and solar ultraviolet irradiation in the wavelength range of 254 nanometers to determine the influence of these space factors on the survival evaluated in terms of colony-forming ability. The results were compared with the results obtained from laboratory experiments simulating these factors.

The following selected space factors affected the survival of the spores in a manner similar to that observed in simulation experiments on the earth. Space vacuum alone did not cause a decrease in survival of

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predried spores. The combined action of space vacuum and solar ultraviolet irradiation in the wavelength range of 254 nanometers resulted in a synergistic effect (that is, an ultraviolet supersensitivity). The additional environmental factors of space and space flight did not influence the viability and irradiation response of the spores remarkably. Therefore, the possibility exists that air-dried spores may survive exposure to space vacuum if shielded against solar irradiation.

INTRODUCTION

A spacecraft may serve as an unintentional vehicle for the transportation of terrestrial microorganisms to other planets. To prevent the contamination of other planets, it is necessary to know exactly the chance of survival of the most resistant microorganisms in the space environment. Special spacecraft sterilization procedures for planetary space missions will meet the planetary quarantine requirements. These requirements for decontamination are based mainly on the results of laboratory experiments in a simulated space environment.

The participation in the Microbial Response to Space Environment Experiment (M191) made it possible to measure the viability of Bacillus subtilis spores that were exposed directly to space environment. The parameters under investigation were space vacuum or space vacuum combined with solar ultraviolet (uv) irradiation at a peak wavelength of 254 nanometers.

BACKGROUND

Data published by different authors on the viability of Bacillus subtilis spores after exposure to ultrahigh vacuum (UHV) show some correspondence (table I). When dried samples kept at atmospheric pressure were taken as controls, the experimental survival values ranged from 70 to 113 percent. The final pressure and the exposure time revealed no observable influence on survival. Nearly the same amount of spores that endured drying procedures at atmospheric pressure survived the exposure to UHV. However, when the colony-forming spores of the primary suspension (initially pipetted on the support) were used as controls, the experimental survival data were much lower and had a greater variance. The preparation method seems to influence the number of survivors. Whereas approximately 15 percent of predried spores exposed on glass sheets survived UHV, exposure of spore suspensions on polyethylene disks covered with polyvinyl alcohol (PVA) resulted in survival rates approximately three times greater than this value.

The mechanism of the biological effect of vacuum has been studied more intensively on Escherichia coli B/r. The vacuum effects observed in stationary-phase cells of this strain are as follows.

1. Damaged membrane (refs. 1 and 2)

    a. Loss of ability

        (1) To form colonies (ref. 3)
        (2) For cellular growth (refs. 4 and 5)
        (3) To produce phage (refs. 4 and 5)
        (4) To respire (refs. 4 and 5)

    b. Repair of cell-membrane damage during prolonged lag phase of subsequent growth (refs. 2 and 6)

2. Increased sensitivity to X-rays (ref. 6)

3. Increased sensitivity to uv rays (refs. 6 and 7)

    a. No photoenzymatic repair of uv-induced lesions (ref. 8)

    b. Little or no excision repair of lethal uv-induced lesions (ref. 8)

    c. Ultraviolet photoproducts

        (1) Pyrimidine dimers (ref. 9)
        (2) Deoxyribonucleic acid (DNA) protein crosslinks (ref. 9)
        (3) Spore-type photoproduct (ref. 9)
        (4) Unidentified thymine-containing product (ref. 9)

4. Decreased sensitivity to heat (ref. 10)

Bacillus subtilis spores also had a uv supersensitivity during vacuum exposure (ref. 10 and fig. 1). The enhancement factor, determined from the dose-survival curves, is 7.7 for the slopes of the linear part of the curves and is 20.0 for the D_{10} values, where D_{10} is the uv dose resulting in a survival of 10 percent (ref. 8). Results of space flight experiments indicated that solar uv irradiation of a wavelength between 200 and 300 nanometers was mainly responsible for the killing of space-exposed microorganisms (refs. 11 to 13). This experiment was conducted primarily on dried T-1 phage. The strong damaging effect of solar uv irradiation in space might be caused by the same photoproducts that have been detected in microorganisms that have been uv irradiated in a high-vacuum environment (ref. 9).

MATERIAL AND METHODS

Biological Object

The Bacillus subtilis strain 168 spores were obtained from Dr. M. Brendel of the Institute of Microbiology at the University of Frankfurt. The incubation on Bacto TAM Sporulation Agar (Difco) at 303° K (30° C) for 20 days resulted in approximately 95-percent sporulation. After resuspension in distilled water, the sample was treated with lysozyme at a concentration of 0.75 mg/cm^3 (0.75 mg/ml) for 4 hours at room temperature to digest the residual vegetative cells. Pure spore suspensions were obtained by washing the sample nine times in cold, distilled water. The spores were stored in suspensions in distilled water (approximately 5×10^{10} spores/cm^3 (5×10^{10} spores/ml)) at 277° K (4° C).

Preflight Preparation Methods

Spore suspensions in cold, distilled water (277° K (4° C)) and cold phosphate-buffered saline (PBS)(277° K (4° C)) were prepared. The suspensions contained 2×10^8 spores/cm^3 (2×10^8 spores/ml). During flight, the samples were kept in cuvettes (cubes of 0.05-cm^3 (50 microliter) capacity) that had quartz windows. The wet samples were prepared at the NASA Lyndon B. Johnson Space Center (JSC) by pipetting aliquots of approximately 0.05 cm^3 (50 microliters) of the suspensions into type A cuvettes. For this purpose, spore suspensions of 100 cm^3 (100 milliliters) each in distilled water and in PBS were transported from Frankfurt, Germany, to the JSC in a protective transporter. The dry samples were exposed on squares (4.8 by 4.8 millimeters) of cover glass. These glass squares had glass bars affixed on two opposing edges to provide space for the bacteria. The total height of each support was 0.8 millimeter. The supports were charged with 0.001 cm^3 (1 microliter) of cold spore suspension in distilled water or in PBS, yielding 2×10^5 spores/support. These samples were dried for 24 hours over silica gel at 277° K (4° C). After drying, the samples were kept at room temperature until they were transported to the JSC, where type B cuvettes (unvented) and type C cuvettes (vented) were loaded with the predried samples.

Flight Experiment Performance

The cuvettes were placed in the microbial ecology evaluation device, the flight hardware on board the Apollo 16 spacecraft. During flight,

the samples were exposed to solar uv irradiation at a peak wavelength of 254 nanometers (three different doses) and to space vacuum for 1.3 hours. In addition, nonirradiated flight controls were used. Ground controls and launch vibration controls were used at the JSC; a simultaneous laboratory simulation experiment, performed at the University of Frankfurt, was exactly parallel to the time schedule of the Apollo 16 flight.

Postflight Analysis

The experiment was received in Frankfurt on May 4, 1972, and was transported by a courier with the biostack experiment (M211) in the biostack ground support equipment (GSE). The temperature in the GSE, indicated by two maximum-minimum thermometers, was in the range of 293° to 298° K (20° to 25° C) during transport. The box containing the cuvettes was sealed with tape. No damage could be detected. The cuvette serial numbers were checked, and the cuvettes were sorted according to the different factors applied, based on the master log list and the experiment plan, respectively.

For the unloading process, the plugs and spacing material of the cuvettes (types B and C) were removed with sterile needles and forceps. Each glass support of cuvettes (types B and C) with dried spores was dropped into a vial containing 1 cm^3 (1 milliliter) of PBS at 277° K (4° C). The fluid contents of each cuvette (type A) were transferred into a vial containing 1 cm^3 (1 milliliter) of PBS. The vials were shaken vigorously for 5 minutes to resuspend the spores homogeneously. The whole suspension, or the appropriate serial dilutions, was spread on Bacto Nutrient Agar (Difco) plates. After incubation for approximately 20 hours at 310° K (37° C), the macrocolonies were counted.

The survival value N/N_0 of the bacterial spores (where N is the number of colony formers after exposure and N_0 is the number of colony formers of the controls) was evaluated in terms of colony-forming ability. The results were compared with those of the laboratory simulation experiment, which was conducted simultaneously in Frankfurt.

RESULTS AND DISCUSSION

The objective of this experiment was to find out whether, during space flight, space vacuum and solar uv irradiation affect the colony-forming ability of Bacillus subtilis spores in a manner similar to that observed in laboratory experiments simulating these factors.

Effect of Space Vacuum

The spores were exposed to space vacuum in two different ways: predried samples from suspension in distilled water (unprotected-exposed spore samples) and predried samples from suspension in PBS (protected-exposed spore samples). In both cases, the spores were spread in monolayers to prevent protection from exposure by being covered.

The survival of these two differently prepared series of samples after exposure to space vacuum is shown in table II. To determine the survival value (N/N_0), the colony formers, after exposure, were related to colony formers in the primary suspension (the dried flight controls) or to those in the dried ground controls. For spores from the distilled-water suspension, a high inactivation value was obtained compared with the colony formers of the primary suspension pipetted onto the support before predrying.

A survival value of approximately 1 was obtained compared with the dried flight controls, and a value of N/N_0 of approximately 5 was obtained in comparison with the dried ground controls. However, a great uncertainty exists in these results because all predried samples from aqueous suspension had an unexpectedly low number of colony formers. In addition, the data are based on only 30 percent of the available cuvettes. The colony formers of the residual cuvettes were each less than 20. These cuvettes were not evaluated because they were assumed to have been subjected to some additional stress not caused by the intended experiment. The reason for the occurrence of this additional inactivation in some of the flight and ground samples is not clear yet.

Postflight laboratory tests of the identical spore suspension were indicative of a survival of 5×10^{-1} after a vacuum exposure of 1 hour to a final pressure of 6.65×10^{-5} N/m^2 (5×10^{-7} torr) when the spores were exposed on a PVA film without predrying. Unfortunately, such PVA supports were not suitable in the space flight experiment because predrying of the samples was necessary. During the slow drying process over silica gel, the PVA dissolved and embedded the spores. The embedded spores remained completely viable after vacuum exposure and even after uv irradiation in vacuum.

The samples of the simulation test conducted at the University of Frankfurt did not show the high inactivation that was observed with the JSC samples, although the survival was still lower by a factor of approximately 10 compared with the results obtained earlier. Furthermore, postflight studies with aqueous suspensions prepared from the same stock suspension again resulted in survival values that were essentially the same as those values usually obtained in the Frankfurt laboratory. The

results of further studies are expected to clarify whether the spores of this special aqueous suspension were especially sensitive to the drying treatment or whether they adhered irreversibly to the support.

In contrast, 8×10^{-1} of the spores from PBS suspension survived space vacuum in relation to the primary suspension. This fact is indicative that the presence of salts during drying increases the survival of the spores. In relation to the two types of predried controls, the survival value was approximately 1. All available cuvettes of this part of the experiment were used for evaluation. The parallel samples had good correspondence (table III). No remarkably different influences were detected among the different ground controls from the PBS suspension.

In both types of exposure (the samples from distilled-water suspension and those from PBS suspension), the survival value in relation to the flight controls was approximately 1. Therefore, the observed inactivation is assumed to be caused by the predrying treatment and not by the additional vacuum exposure. This observation is in agreement with laboratory findings obtained earlier (refs. 10 and 14 to 19).

In suspension, the number of spores remaining viable during flight was identical to those of the ground controls (table III). This fact is true for suspensions in distilled water and in PBS. When compared with the samples remaining in Frankfurt, a decrease of colony-forming ability was observed for both suspensions (2×10^{-1} in aqueous suspension and 3×10^{-1} in PBS suspension). Because of the good correspondence of all flight and ground controls in each series, it is apparent that the additional environmental factors of space and space flight exerted no significant influence on the colony-forming ability of Bacillus subtilis spores.

Effect of Solar Ultraviolet Irradiation on Spores in Suspension

The survival value of spores in suspension irradiated with solar uv irradiation during space flight is in good agreement with the corresponding values of the dose-survival curve obtained by uv irradiation of the suspension during the simulation test in Frankfurt (fig. 2). This agreement is indicative that the additional space flight factors did not remarkably influence the uv sensitivity of bacterial spores in suspension.

Combined Effect of Solar Ultraviolet Irradiation and Space Vacuum

Simultaneous exposure to space-vacuum environment was expected to increase the sensitivity of spores to uv irradiation, compared with the

uv sensitivity of spores irradiated at atmospheric pressure. This assumption is probable because of the results of earlier laboratory experiments of uv irradiation during vacuum exposure (ref. 10). The data obtained support this assumption (fig. 3). Spores irradiated with solar uv irradiation in space-vacuum environment had an increased uv sensitivity, although just one of the three flight samples was in good agreement with the samples of the simulation test (uv irradiated in high vacuum). The apparent lessened supersensitivity of the two other samples may be caused by the high variance of the nonirradiated samples.

CONCLUDING REMARKS

The effect of two specific space factors on the colony-forming ability of Bacillus subtilis spores was tested in this experiment. These factors were space vacuum and ultraviolet irradiation at a wavelength of 254 nanometers. Additionally, the spores were exposed to several random space flight factors, such as launch vibration, weightlessness, and gas composition in the Apollo command module. Any influence of these additional space flight factors on the viability of the spores was not detected.

Predried samples were used for exposure to space vacuum. A high level of inactivation of spores from suspensions in distilled water and less inactivation of spores from suspension in phosphate-buffered saline were the results of the predrying procedure. This degree of inactivation was not changed by an additional exposure to space vacuum. Thus inactivation seems to occur during water desorption. Therefore, spores surviving air drying, possibly because of the presence of protecting substances, may have an augmented capability to retain viability during exposure to space vacuum.

The ultraviolet sensitivity of wet spores that were exposed in suspension in distilled water to solar ultraviolet irradiation during space flight was exactly the same as that determined for the ground simulation experiments. Simultaneous exposure to space vacuum increases the ultraviolet sensitivity of the spores, in accordance with the results of the simulation experiment. This supersensitivity to solar irradiation of microbes in space reduces the chance for living forms to pass through space undamaged and, consequently, to contaminate other planets.

However, if a possibility of shielding against solar irradiation exists (for example, by minerals or soil), air-dried spores may survive the exposure to the harsh space environment. Therefore, the requirements for spacecraft sterilization are necessary to prevent the transport of viable terrestrial microbes to other planets.

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TABLE I.- SURVIVAL OF BACILLUS SUBTILIS SPORES THAT WERE EXPOSED TO UHV AT ROOM TEMPERATURE

Exposure conditions						Survival, percent		
Pressure		Time, day	Primary suspension	Pretreatment	Support	Relative to primary suspension	Relative to dried samples at 1 atm	Reference number
N/m^2	Torr							
1.33×10^{-4}	1×10^{-6}	32	Peptone solution, 2 percent	Lyophilized	Tubes	+	None	20
1.04×10^{-6} 7.80×10^{-7}	8×10^{-9} 6×10^{-9}	10 45	Aqueous	None	Tubes	+ -	None	21
7.80×10^{-7}	6×10^{-9}	35	Aqueous	None	Tubes	50	100	14
6.5×10^{-7}	5×10^{-9}	5	Aqueous	Predried	Glass fiber	-	113	15
6.5×10^{-7}	5×10^{-9}	7	Aqueous	Predried	Glass sheets	15	107	10
1.33×10^{-7}	1×10^{-9}	3	Aqueous	Predried	Filter paper	36	70	16
5.2×10^{-8}	4×10^{-10}	5	Aqueous	None	Filter paper	-	92	17
1.33×10^{-8}	1×10^{-10}	14	Aqueous	Predried	Metallic planchets	-	90	18
9.1×10^{-9}	7×10^{-11}	20	Aqueous	Predried	Glass sheets	12	86	19

TABLE II.- SURVIVAL OF *BACILLUS SUBTILIS* SPORES AFTER EXPOSURE TO SPACE VACUUM FOR 1.3 HOURS

Predried samples prepared from suspension in —	Flight survival value (N/N_0) in relation to —		
	Primary suspension	Dried flight controls	Dried ground controls
Distilled water	6.6×10^{-3}	1.4	4.3
PBS	$(8.1 \pm 0.3) \times 10^{-1}$	1.1 ± 0.1	1.0 ± 0.1

TABLE III.- COLONY-FORMING SPORES OF BACILLUS SUBTILIS EXPOSED IN A DIFFERENT MANNER TO DIFFERENT FACTORS OF SPACE AND SPACE FLIGHT

Type of exposure	Applied factor	Type of stock suspension	Colony formers/cuvette			
			Flight samples	Ground controls	Vibration controls	Simulation test
Predried	Vacuum	Distilled water	9.25×10^2	None	None	$(1.65 \pm 0.89) \times 10^3$
		PBS	$(1.19 \pm 0.05) \times 10^5$	None	None	$(9.20 \pm 4.00) \times 10^4$
	1 atm	Distilled water	6.62×10^2	1.80×10^2	$(2.45 \pm 1.10) \times 10^2$	$(2.76 \pm 2.69) \times 10^3$
		PBS	$(1.08 \pm 0.05) \times 10^5$	$(1.15 \pm 0.18) \times 10^5$	$(1.22 \pm 0.18) \times 10^5$	$(7.40 \pm 2.00) \times 10^4$
Suspension	1 atm	Distilled water	$(1.89 \pm 0.67) \times 10^6$	$(1.89 \pm 0.27) \times 10^6$	$(2.33 \pm 0.26) \times 10^6$	8.35×10^6
		PBS	$(3.21 \pm 0.19) \times 10^6$	$(3.00 \pm 0.20) \times 10^6$	$(3.83 \pm 0.16) \times 10^6$	9.10×10^6

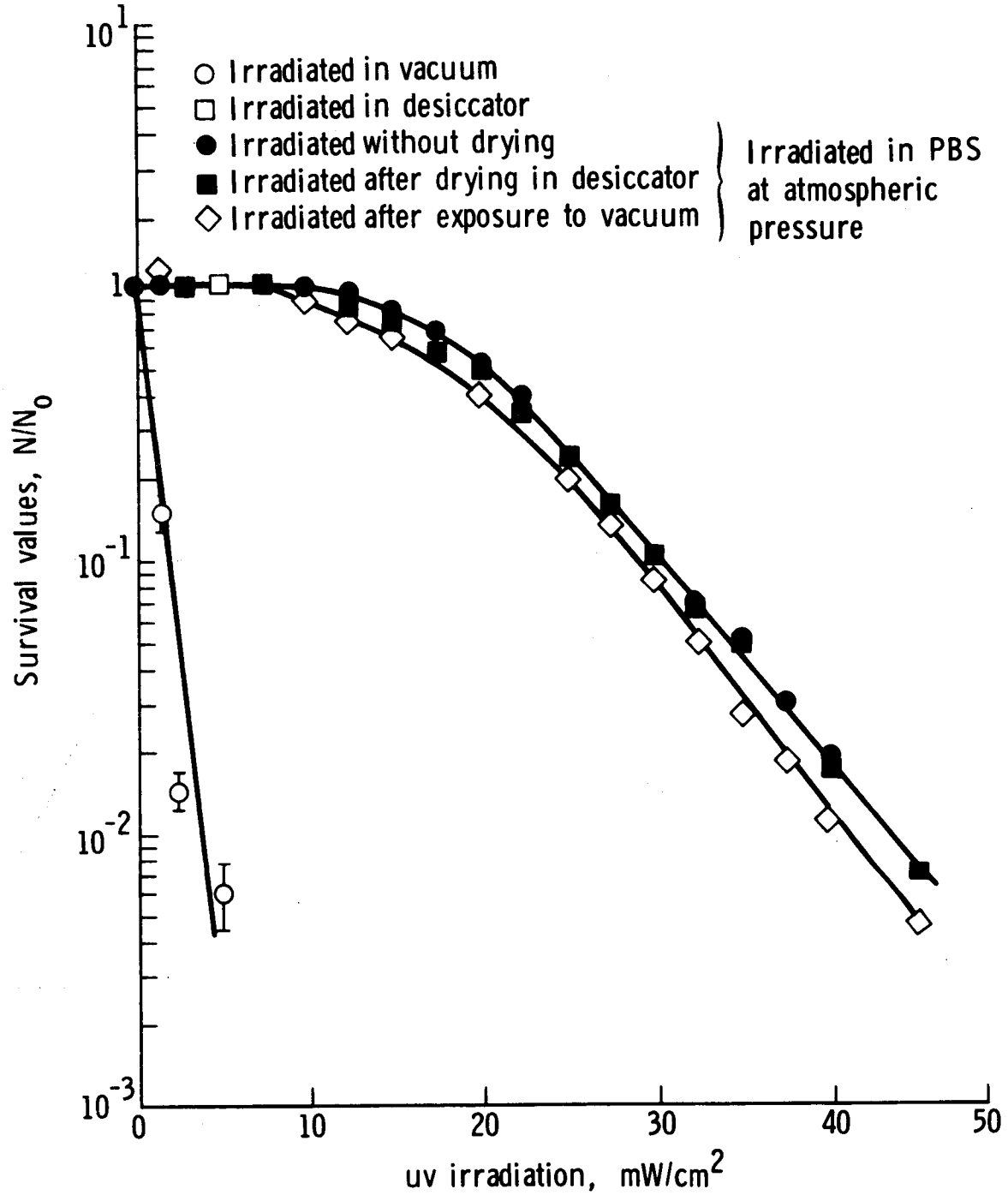

Figure 1.- Survival of Bacillus subtilis spores irradiated with uv of 254 nm at atmospheric pressure in suspensions, after desiccator drying, or in a 1.33×10^{-4}-N/m^2 (10^{-6} torr) vacuum.

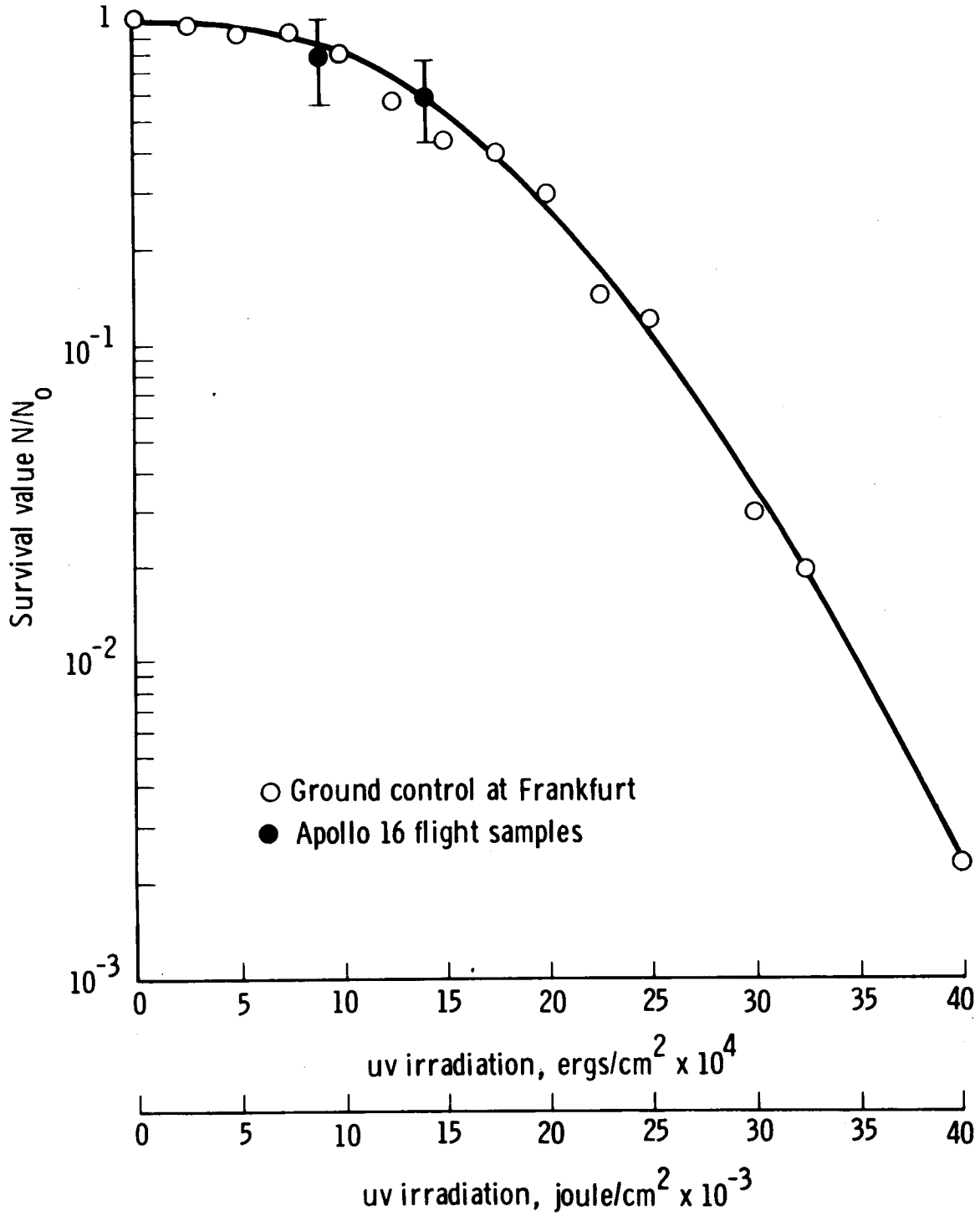

Figure 2.- Survival of Bacillus subtilis spores in suspensions of distilled water irradiated with solar uv of 254 nm during space flight or with uv of 254 nm on the ground.

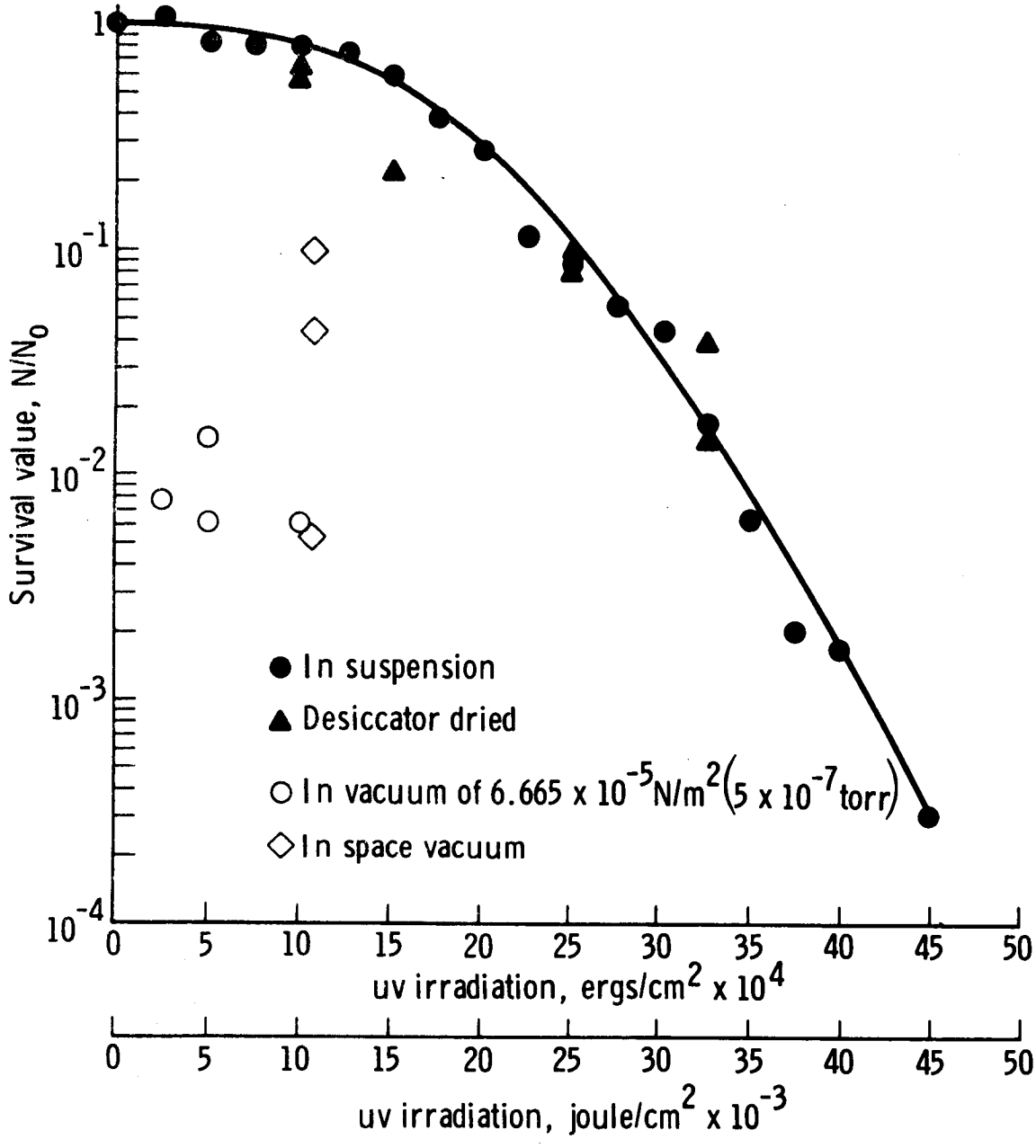

Figure 3.- Survival of Bacillus subtilis spores irradiated with solar uv of 254 nm in space vacuum or with uv of 254 nm in vacuum during the simulation test.

POSTFLIGHT ANALYSES OF BACILLUS THURINGIENSIS ORGANISMS EXPOSED TO SPACE FLIGHT CONDITIONS

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ABSTRACT

Bacillus thuringiensis var. *thuringiensis* spores flown in the Apollo 16 Microbial Response to Space Environment Experiment (M191) were examined for viability and alterations in toxin production capability. Although exposure of the spores to full sunlight resulted in significant reduction in viability, exposure of the spores to ultraviolet irradiation at peak wavelengths of 254 and 280 nanometers did not produce differences in survival rates. Statistically, significant differences in the capability of the postflight isolates to produce toxins were not found among the treatment groups.

SUMMARY

Cultures of *B. thuringiensis* returned from space flight appeared to be normal to slightly affected adversely in their ability to produce three toxins that affect insects. In addition, it can be stated that *B. thuringiensis* spores are very resistant to ultraviolet irradiation at the individual wavelengths and energy levels previously described. Full sunlight, however, does have a detrimental effect on the viability of *B. thuringiensis* spores.

INTRODUCTION

An objective of the Microbial Response to Space Environment Experiment (M191) was to test the effects of space flight conditions on selected biological systems. *B. thuringiensis* was selected for inclusion in this experiment in an effort to determine how such conditions might affect

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microbial toxin production. B. thuringiensis var. thuringiensis (serotype I) produces at least three toxins that affect insects: α-exotoxin, a Phospholipase C resembling in some ways the Phospholipase C produced by Clostridium perfringens; β-exotoxin, a nucleotide that is heat stable and kills insects at time of molt or pupation (that is, at times of heavy cell mitosis); and δ-endotoxin, a protein that destroys the midgut cells in many Lepidopterans within 20 minutes of ingestion, causing gut paralysis and eventual death (ref. 1).

MATERIALS AND METHODS

The microorganism used for this experiment was Bacillus thuringiensis var. thuringiensis 996TC supplied by the Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland. Spores were obtained by growing the microorganism on AK agar (Baltimore Biological Laboratory) slants for 4 to 5 days at 303° K (30° C). The spores were washed from the slants with sterile, distilled water and were heat shocked for 10 minutes at 333° K (60° C) to kill any remaining vegetative cells. After three successive washes, the spore pellet was suspended in distilled water at a final concentration of 6.4×10^8 spores/cm^3 (6.4×10^8 spores/ml). Aliquots of 1-cm^3 (1 ml) of the spore suspensions were then drawn through Millipore filter pads, resulting in a monolayer of spores on the filter. Then, the spore-impregnated filter was cut into chips 0.5 by 0.5 centimeter square that, in turn, were loaded into type B (dry) cuvettes. In addition to the various inflight stresses (that is, shock, vibration, and increased g forces during launch and recovery; reduced gravity; altered geophysical forces; and cosmic irradiation), the organisms in 32 of the 41 inflight experiment cuvettes were exposed to various levels of solar irradiation for a period of 10 minutes (table I). Nine cuvettes were held as ground controls and nine were employed as vibration controls and, as such, were exposed to simulated launch vibrations at the NASA Lyndon B. Johnson Space Center (JSC).

Upon return of the experiment hardware, the spore-impregnated Millipore filter chips were unloaded from their cuvettes and placed in 1.0 cm^3 (1.0 milliliter) of distilled water at pH 6.0 for 24 hours. This treatment was followed by mild sonication to facilitate removal of the spores from the filter pads. After serial dilution, 0.1-cm^3 (0.1 milliliter) aliquots of each spore suspension were spread onto sheep blood agar plates and maltose agar plates in quadruplicate. All plates were incubated at 303° K (30° C) for 24 hours. The sample-plating scheme used throughout this experiment is shown in figure 1.

Colonies growing on sheep blood agar plates were counted to determine survival and were analyzed for the presence of any abnormal morphology. In addition, colonies were examined for zones of β-hemolysis that deviated from the controls. All abnormal colonies and 10 percent of the normal colonies were transferred to maltose agar plates and to Sarcina flava inhibition plates.

Also, colonies growing on maltose agar were observed for abnormal colony morphology and the ability to use maltose (exemplified by a yellow zone surrounding each colony). All abnormal colonies and 10 percent of the normal colonies were transferred to sheep blood agar plates and S. flava inhibition plates.

The S. flava inhibition plates were observed for the production of β-exotoxin, exemplified by a zone of inhibition surrounding colonies of B. thuringiensis (ref. 2). Zones of inhibition were compared with those of the control groups. Any colony that was surrounded by a zone that deviated from the control standard was subjected to further quantitative analysis by using the housefly assay system (ref. 3).

The housefly assay system consisted of spotting 1 cm^3 (1 milliliter) of the serially diluted toxin preparation onto circular filter pads fitted into 20- by 60-millimeter Petri dishes. Each dilution was seeded with 10 late third instar housefly larvae in duplicate and held at room temperature ($\cong$ 298° K) until all controls had emerged and died. Then, each dilution was scored for the number of deaths, number of emerging adults, and the number of mutants.

The abnormal isolates also were submitted to tests for α-exotoxin and δ-endotoxin production. The test for α-exotoxin consisted of measuring phospholipase activity on egg yolk agar plates (ref. 4).

To encourage sporulation, the organisms were cultured on tryptose phosphate agar with manganese sulphate added at the rate of 2 ng/cm^3 (2 ng/ml). Duplicate plates were made for spore-crystal production. These plates were incubated at 305° K (32° C) for 72 hours. The plate cultures were washed off the agar with sterile saline after a nigrosin smear was made of each culture. Then, the suspended spores and crystals were adjusted in a colorimeter to give the same turbidity. By use of a Dutky-fest microinjector, precise amounts of each culture suspension were injected into the midguts of 15 third instar silkworm larvae, and these larvae were held in Petri plates and fed ad libitum for 24 hours, at which time deaths were recorded. A total of 3250 insects was injected.

RESULTS

A statistical quantitative analysis of B. thuringiensis survivors for each treatment group was performed. There appears to have been no significant difference between the mean of B. thuringiensis survivors from the ground control, flight control, and vibration control units. Also, there was no significant difference between the means of B. thuringiensis survivors for any of the 254- and 280-nanometer-exposed groups. There was a significant difference ($P \leq 0.01$) in those survivors from the full-light-exposed cuvettes compared with the nonirradiated control groups (table II).

The S. flava assay plates produced 18 isolates that were considered to be abnormal with respect to β-exotoxin production. The isolates were obtained from the following control and exposure groups: ground control, 5; flight control, 1; vibration control, 3; and flight exposed, 9 (6 at 254 nanometers, 3 at 280 nanometers and 0 at full light). Analyses of these isolates using the housefly assay system indicated that they did possess altered β-exotoxin production characteristics (table III).

The resistant nature of B. thuringiensis spores again is illustrated by the relative lack of β-exotoxin mutants isolated from 254- and 280-nanometer-exposed cuvettes. In fact, the abnormal isolates recovered appear to be distributed randomly throughout control and exposed groups. The absence of the ability to produce β-exotoxin was absent in isolate 6603; however, this isolate was retrieved from a ground control cuvette. The results of the housefly assay tests on the morphologically abnormal isolates and on a representative number of normal isolates is presented in table IV. The irradiated cultures appeared generally to have been affected slightly in their ability to produce the α-exotoxin Phospholipase C (table V). However, whether these differences are significant is questionable. One unexpected event was the loss of the ability to produce Phospholipase C by the ground-based control isolate 6603, the same isolate that lost its ability to produce β-exotoxin.

Because of the short time available to conduct the analyses for δ-endotoxin production, it was necessary to establish one set of dilutions and begin the experiment. It was hoped that the dilutions selected would produce lethality between LD_{10} and LD_{90} (10- to 90-percent kill) so that probit analysis could be conducted. Unfortunately, the dilutions selected were too toxic, and only the lowest doses permitted survival (table VI). Nevertheless, the experiment was indicative that all cultures were toxic, although a critical comparison with the controls is not possible. There was slight increase in toxicity by some of the irradiated cultures, and there was an apparent loss of virulence in others. These slight differences probably are not valid, and none of the irradiated cultures changed significantly in virulence for the silkworm.

DISCUSSION

An evaluation of the survival data seems to be suggestive that B. thuringiensis spores are somewhat resistant to irradiation at wavelengths of 254 and 280 nanometers. In those cuvettes exposed to full sunlight, a decreased survival was observed. Previous terrestrial studies have been indicative that B. thuringiensis spores are very susceptible to full sunlight (ref. 5). Although it is possible that a particular wavelength or combination of wavelengths is responsible for the reduced spore viability, the data presented here are insufficient to make such a conclusion.

It is of particular interest that the ground control isolate 6603 lost its ability to produce both α- and β-exotoxin. This is a most unusual event because production of these two materials, one a protein and one a nucleotide, is not likely to be related genetically.

In general, the relative lack of alterations in the toxin-producing capability of B. thuringiensis organisms flown in this experiment would seem to imply that space flight stresses are insufficient to cause such changes. However, the total knowledge of microbial response to space flight stress is inadequate to permit extrapolation of these data to other toxin-producing microorganisms.

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TABLE I.- BACILLUS THURINGIENSIS WAVELENGTH AND ENERGY EXPOSURE PATTERN

Number of cuvettes	Bandpass filter type, peak wavelength, nm	Total energy received	
		Joules/cuvette	Ergs/cuvette
3	254	4.0×10^{-5}	4.0×10^{2}
3	254	4.0×10^{-4}	4.0×10^{3}
3	254	1.0×10^{-3}	1.0×10^{4}
3	254	4.0×10^{-3}	4.0×10^{4}
3	280	5.0×10^{-5}	5.0×10^{2}
3	280	5.0×10^{-4}	5.0×10^{3}
3	280	1.0×10^{-3}	1.0×10^{4}
3	280	4.0×10^{-3}	4.0×10^{4}
1	Full light	1.5×10^{-2}	1.5×10^{5}
1	Full light	1.9×10^{-1}	1.9×10^{6}
2	Full light	1.5×10^{2}	1.5×10^{7}
1	Full light	3.4×10^{2}	3.4×10^{7}
3	Full light	1.4×10^{1}	1.4×10^{8}

TABLE II.- COMPARISONS AMONG MEANS OF THE LOGARITHMIC TRANSFORMATION OF THE NUMBER OF *BACILLUS THURINGIENSIS* SURVIVORS

Test groups	Mean	At full light	At 280 nm	At 254 nm	Ground control	Vibration control
Flight control	6.777 69	[a]2.421 35	0.865 06	0.641 60	0.124 92	0.005 96
Vibration control	6.771 73	[a]2.415 39	.859 10	.635 63	.118 96	
Ground control	6.652 77	[a]2.296 43	.740 14	.516 67		
At 254 nm	6.136 09	1.779 76	.223 47			
At 280 nm	5.912 63	1.556 29				
At full light	4.356 34					

[a]Indicates a difference in the means greater than the 95-percent critical value of 1.83.

TABLE III.- RECORD OF TOXICITY OF BACILLUS THURINGIENSIS β-EXOTOXIN (FLY TOXIN) FOR LAST INSTAR HOUSEFLY LARVAE (MUSCA DOMESTICA) - ISOLATES WITH ABNORMAL TOXIN PRODUCTION IN SARCINA FLAVA TESTS

Culture	Irradiation treatment energy		Larval mortality, percent	
	Joules	Ergs	Toxin concentration = 1 mg/cm^3	Toxin concentration = 0.1 mg/cm^3
At 280-nm wavelength				
7210	5.9×10^{-4}	5.9×10^{3}	20	20
7230-A	5.9×10^{-4}	5.9×10^{3}	45	10
7230-B	5.9×10^{-4}	5.9×10^{3}	20	0
At 254-nm wavelength				
7205-A	3.2×10^{-5}	3.2×10^{2}	85	35
7205-B	3.2×10^{-5}	3.2×10^{2}	75	10
7228	3.5×10^{-4}	3.5×10^{3}	100	55
7215-A	8.7×10^{-4}	8.7×10^{3}	55	40
7215-B	8.7×10^{-4}	8.7×10^{3}	45	10
7248	9.7×10^{-4}	9.7×10^{3}	90	45
Controls (a)				
7252	Vibration control		100	35
7257	Vibration control		30	25
7270	Vibration control		100	10
6603	Ground control		10	0
7220-A	Ground control		25	30
7220-B	Ground control		30	15
7220-C	Ground control		20	15
7264	Ground control		100	15
6619	Flight control		65	10
None	Preflight control		100	45

[a]None of the controls were treated with irradiation.

TABLE IV.- RECORD OF TOXICITY OF BACILLUS THURINGIENSIS β-EXOTOXIN (FLY TOXIN) FOR LAST INSTAR HOUSEFLY LARVAE (MUSCA DOMESTICA) - MORPHOLOGICALLY NORMAL AND ABNORMAL ISOLATES

Culture	Irradiation treatment energy		Mortality (adjusted for controls), percent			
			Toxin concentration			
	Joules	Ergs	5 mg/cm^3	0.5 mg/cm^3	0.05 mg/cm^3	0.005 mg/cm^3
At full sunlight						
6622-II	1.5×10^{6}	1.5×10^{7}	100	100	24	0
6608-I	1.4×10^{1}	1.4×10^{8}	100	100	52	0
6608-II	1.4×10^{1}	1.4×10^{8}	100	100	8	0
At 280-nm wavelength						
7210-I	5.9×10^{-4}	5.9×10^{3}	100	100	73	0
7210-II	5.9×10^{-4}	5.9×10^{3}	100	63	0	0
7230-A	5.9×10^{-4}	5.9×10^{3}	100	85	2	0
7230-B	5.9×10^{-4}	5.9×10^{3}	100	100	15	0
7202	5.9×10^{-4}	5.9×10^{3}	100	0	0	0
7204-II	6.1×10^{-4}	6.1×10^{3}	100	100	21	0
7203-I	1.2×10^{-3}	1.2×10^{4}	100	98	0	0
7203-II	1.2×10^{-3}	1.2×10^{4}	100	100	15	0
7227-I	6.5×10^{-3}	6.5×10^{4}	100	100	74	0
7227-II	6.5×10^{-3}	6.5×10^{4}	100	100	24	0
At 254-nm wavelength						
7242-I	3.2×10^{1}	3.2×10^{8}	100	98	4	0
7242-II	3.2×10^{-5}	3.2×10^{2}	100	100	96	0
7205-A	3.2×10^{-5}	3.2×10^{2}	100	100	5	0
7205-B	3.2×10^{-5}	3.2×10^{2}	100	100	81	0
7218-I	3.5×10^{-4}	3.5×10^{3}	100	95	0	0
7218-II	3.5×10^{-4}	3.5×10^{3}	100	82	10	0
7228	3.5×10^{-4}	3.5×10^{3}	100	100	56	4
7241-I	8.7×10^{-4}	8.7×10^{3}	100	98	6	0
7241-II	8.7×10^{-4}	8.7×10^{3}	100	100	84	0
7215-A	8.7×10^{-4}	8.7×10^{3}	97	97	8	0
7208-I	9.7×10^{-4}	9.7×10^{3}	100	95	0	0
7208-II	9.7×10^{-4}	9.7×10^{3}	100	100	71	0
7248	9.7×10^{-4}	9.7×10^{3}	100	100	52	0

TABLE IV.- RECORD OF TOXICITY OF BACILLUS THURINGIENSIS β-EXOTOXIN (FLY TOXIN) FOR LAST INSTAR HOUSEFLY LARVAE (MUSCA DOMESTICA) - MORPHOLOGICALLY NORMAL AND ABNORMAL ISOLATES - Concluded

Culture	Irradiation treatment energy		Mortality (adjusted for controls), percent			
			Toxin concentration			
	Joules	Ergs	5 mg/cm^3	0.5 mg/cm^3	0.05 mg/cm^3	0.005 mg/cm^3
Controls (a)						
7252	Vibration control		100	100	49	0
7257	Vibration control		100	100	27	0
996	Beltsville control		100	100	96	10
6603	Ground control		4	2	1	0
6619	Flight control		100	100	45	0

[a]None of the controls were treated with irradiation.

TABLE V.- RECORD OF ZONE WIDTH CAUSED BY PHOSPHOLIPASE C PRODUCED BY TREATED AND CONTROL CULTURES OF BACILLUS THURINGIENSIS

Culture	Irradiation treatment energy		Zone width	
	Joules	Ergs	Range, mm	Average, mm
At full sunlight				
6622-II	1.5×10^{0}	1.5×10^{7}	0.90 to 1.50	1.13
6608-I	1.4×10^{1}	1.4×10^{8}	.90 to 1.30	1.10
6608-II	1.4×10^{1}	1.4×10^{8}	1.10 to 1.20	1.12
At 280-nm wavelength				
7210-I	5.9×10^{-4}	5.9×10^{3}	0.70 to 1.50	1.15
7210-II	5.9×10^{-4}	5.9×10^{3}	.70 to 1.10	.92
7230-A	5.9×10^{-4}	5.9×10^{3}	.80 to 1.20	1.02
7230-B	5.9×10^{-4}	5.9×10^{3}	.80 to 1.20	.95
7202	5.9×10^{-4}	5.9×10^{3}	.90 to 1.70	1.40
7204-II	6.1×10^{-4}	6.1×10^{3}	.90 to 1.70	1.20
7203-I	1.2×10^{-3}	1.2×10^{4}	1.00 to 1.30	1.12
7203-II	1.2×10^{-3}	1.2×10^{4}	.90 to 1.10	1.00
7227-I	6.5×10^{-3}	6.5×10^{4}	.80 to 1.40	1.05
7227-II	6.5×10^{-3}	6.5×10^{4}	.80 to 1.20	.93
At 254-nm wavelength				
7242-II	3.2×10^{-5}	3.2×10^{2}	1.10 to 1.20	1.18
7205	3.2×10^{-5}	3.2×10^{2}	1.10 to 1.50	1.20
7218-I	3.5×10^{-4}	3.5×10^{3}	.90 to 1.20	1.10
7218-II	3.5×10^{-4}	3.5×10^{3}	.50 to 1.20	.87
7228	3.5×10^{-4}	3.5×10^{3}	.90 to 1.40	1.10
7241-I	8.7×10^{-4}	8.7×10^{3}	.70 to 1.10	.88
7241-II	8.7×10^{-4}	8.7×10^{3}	1.00 to 1.30	1.15
7215-A	8.7×10^{-4}	8.7×10^{3}	.70 to 1.90	.90
7208-I	9.7×10^{-4}	9.7×10^{3}	.70 to 1.10	.92
7208-II	9.7×10^{-4}	9.7×10^{3}	1.10 to 1.50	1.15

TABLE V.- RECORD OF ZONE WIDTH CAUSED BY PHOSPHOLIPASE C PRODUCED BY TREATED AND CONTROL CULTURES OF BACILLUS THURINGIENSIS - Concluded

Culture	Irradiation treatment energy		Zone width	
	Joules	Ergs	Range, mm	Average, mm
At 254-nm wavelength - Concluded				
7248	9.7×10^{-4}	9.7×10^{3}	1.00 to 1.50	1.17
7242-I	3.2×10^{1}	3.2×10^{8}	.80 to 1.30	1.03
7205-A	3.2×10^{1}	3.2×10^{8}	1.10 to 1.50	1.20
Controls (a)				
7252	Vibration control			
7257	Vibration control		0.90 to 1.40	1.15
996	Ground control (Beltsville)		1.00 to 1.60	1.25
6603	Ground control (Houston)		No phospholipase produced	No phospholipase produced
6619	Flight control		.70 to 2.00	1.20

[a]None of the controls were treated with irradiation.

TABLE VI.- RECORD OF MORTALITY OF THIRD INSTAR SILKWORM LARVAE THAT WERE FED TREATED AND CONTROL CULTURES OF BACILLUS THURINGIENSIS

[Crystal toxin at 24 hours postfeeding, 15 larvae per group]

Culture	Individual mortalities by estimated weight of toxin, μg					
	20	40	100	200	300	400
At full sunlight						
7204-I	0	0	12	15	15	15
6622-II	0	1	8	15	15	15
6608-I	1	2	13	15	15	15
6608-II	4	5	15	15	15	12
At 280-nm wavelength						
7210-I	2	5	14	13	15	15
7210-II	0	8	13	14	14	15
7230-A	1	7	14	14	15	15
7230-B	2	1	5	13	11	15
7202	0	2	15	15	15	15
7204-II	3	9	14	15	15	15
7203-I	1	1	7	10	10	15
7203-II	0	2	13	15	14	15
7227-I	2	10	15	15	15	15
7227-II	0	4	15	15	15	15
At 254-nm wavelength						
7242-II	2	3	11	14	15	15
7205	5	10	14	15	15	15
7218-I	2	12	13	15	15	15
7218-II	4	7	13	15	15	15

TABLE VI.- RECORD OF MORTALITY OF THIRD INSTAR SILKWORM LARVAE THAT WERE FED TREATED AND CONTROL CULTURES OF BACILLUS THURINGIENSIS - Concluded

[Crystal toxin at 24 hours postfeeding, 15 larvae per group]

Culture	Individual mortalities by estimated weight of toxin, μg					
	20	40	100	200	300	400
At 254-nm wavelength - Concluded						
7228	0	3	15	15	15	15
7241-I	1	0	12	15	15	15
7241-II	0	4	13	15	15	15
7215-A	0	4	14	15	15	15
7208-I	0	1	13	14	10	14
7208-II	0	1	7	9	13	13
7248	2	13	15	14	15	15
7242-I	0	10	15	15	14	15
7205-A	0	2	8	15	15	15
Vibration control						
7252	0	0	13	13	15	15
7257	0	4	15	15	15	15
Ground control						
996	0	2	15	15	14	15
6603	1	0	12	15	15	15
Flight control						
6619	0	3	13	15	15	15

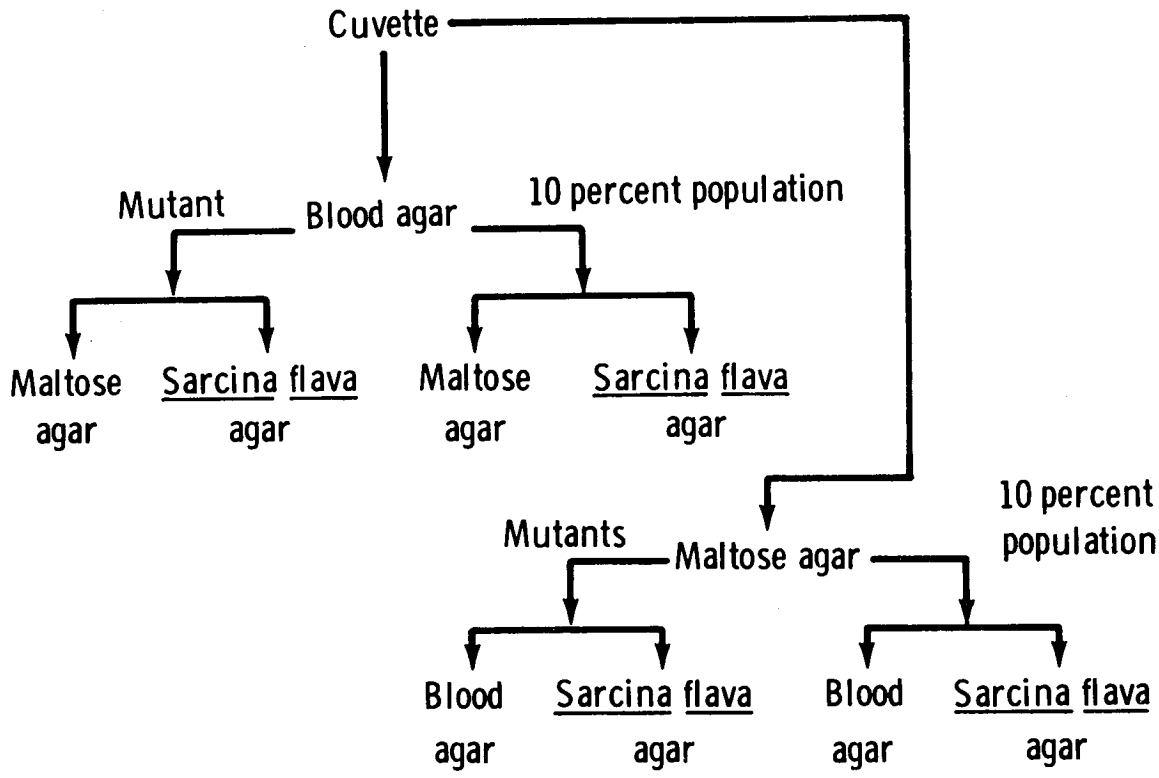

Figure 1.- Bacillus thuringiensis sample-plating scheme.

MYCOLOGICAL STUDIES HOUSED IN THE APOLLO 16 MICROBIAL ECOLOGY EVALUATION DEVICE

By Paul A. Volz*

ABSTRACT

Four fungal species were selected as the mycological test systems for the Microbial Ecology Evaluation Device Experiment (M191) flight hardware for the Apollo 16 mission. Preflight studies and evaluations of diverse fungal species and planned postflight studies were the basis of species selection. Studies on the returned flight fungi were selected in disciplines that were medically associated with man in space.

SUMMARY

Survival, death, and phenotype count have yielded variation in the number of fungi recovered from the controls and the flight-exposed cuvettes during preliminary analysis of postflight first-phase data. Also, the preliminary analysis was indicative that fungi exposed to specific space flight conditions demonstrated variable survival rates and phenotype counts. Specific space flight conditions included full-light space exposure for Chaetomium globosum, exposure at 300- and 254-nanometer wavelengths for Rhodotorula rubra, full-light and 280-nanometer-wavelength exposure for Trichophyton terrestre, and 254-nanometer-wavelength exposure for Saccharomyces cerevisiae. In general, phenotype counts for flight cuvettes and survival rates for control cuvettes were higher compared with the remaining cuvettes.

INTRODUCTION

The fungi provide a large species reserve for the selection of studies in medically related fields applicable to man in space. The fungal species serve as tools for identifying cellular-level change

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incurred in space. One change in the microorganisms could cause death, whereas another change could produce temporary or permanent variation in nutritional requirements, growth dynamics, pathogenicity, cytogenetics, morphology, or biochemical activities. In a closed environmental system such as the unit that houses man in space, the change incurred in the fungal species in flight could produce direct interactions with man. The isolate may use organic or inorganic materials not normally selected for support of growth or other cellular variation.

In the first postflight phase of mycology experimentation in the Microbial Response to Space Environment Experiment (M191), an attempt will be made to identify survival according to exposure to specific quantitative space flight factors. Selected diverse phenotypes subjected to the second phase of studies will result in the identification of change other than cell survival or death. The changes in the microorganisms under study relate to the comfort, safety, and health of the crewmen.

METHODS

The original isolated strains of the four flight fungi and preflight test organisms were collected in their natural habitats by several specialists and were identified by use of key characteristics. The selected strains have been used by other investigators as test systems in various experiments. Strains are cataloged formally and are housed in private or industrial stock reference collections.

Species were selected from the major groups of fungi in tests to determine the microorganisms that are best suited for stowage in the space flight hardware (refs. 1 and 2). Preflight viability studies and ultraviolet (uv) irradiation studies were conducted on 23 species that represent five major fungal classes (table I). For postflight evaluations, mycological studies that incorporated test parameters of the flight hardware also were considered. A review of the literature on space-related research in mycology was used as an aid in the selection process (ref. 3). Anatomical characteristics of organisms limited the selection of several taxa because of the size limitations and lack of survival within the prescribed constraints of the flight hardware.

The survival of preflight test fungi in holding fluids and in dry conditions was determined by the use of longevity studies. Conidia, ascospores, yeast cells, and encysted zoospores were housed in 0.05-cm^3 (0.05 milliliter) volume fluids under anaerobic conditions or were dried on filter paper. Then, these preparations were held for periods equivalent to the time required for loading and unloading flight hardware and for the duration of the actual space flight. Species selected for the Apollo 16 flight have remained viable under flight storage

conditions for 2-1/2 years. Fungal cells stored in distilled water or housed dry retained high viabilities, whereas cells stored in saline or Tween 80 at various pH readings had lower survival rates over prolonged storage periods. Distilled water was selected as the holding fluid for the flight fungi in the wet cuvettes, and additional cuvettes housed dried fungal cells. The species selected for the Apollo 16 flight included the filamentous fungi C. globosum (6205) and T. terrestre (x285) and the yeasts R. rubra (y1592) and S. cerevisiae (y2439).

Fungi in the flight hardware were exposed to different energy levels of uv irradiation at wavelengths of 254, 280, and 300 nanometers; to full light; and to dark (tables II and III). Cuvettes from the Apollo 16 flight experiment, which included cuvettes housed in the vented-exposed tray, unvented-exposed tray, flight control tray, ground control tray, and vibration control tray, were unloaded systematically. The retrieved fungal cells were placed in a dilution series for viability, survival, and phenotypic selection studies. The single-cell phenotype isolates were selected by colony development changes, which included variation in growth rates, sporulation, pigmentation, texture, density, and perimeter.

Mature ascospores, yeast cells, and conidia were harvested from Petri-plate or test-tube-slant colonies of the respective flight genera. Cell suspensions were made using sterile, distilled water. Wet cuvettes were filled with a diluted suspension of the fungal cells (10^4 or 10^5 cell counts/0.05-cm^3 (0.05 milliliter) cuvette volume, depending on the cell size of the respective species). Suspensions of the four flight fungi were filtered for stowage in the dry cuvettes. Filter paper squares with attached fungal cells were cut with a square punch and were placed in the dry cuvettes. To reduce cell loss because of static electricity, filter paper squares were placed in the cuvettes before the squares were dry.

A Tween 80 solution was used to wet the filter paper squares before removal and to syringe-wash both the wet- and dry-cuvette interiors. The wetting agent was used to dislodge fungal cells during postflight cuvette unloading. All procedures were conducted aseptically.

RESULTS

Immediately after unloading cuvettes for the initiation of postflight studies, cell aliquots were used to determine survival rates in relation to exposure to the selected space flight parameters and in relation to the controls. Some variation occurred among cells stored in

wet (type A), dry unvented (type B), or dry vented (type C) cuvettes. However, variation in survival among cells stored in wet or dry conditions remained in proportion to the cells of the four fungal species that were stored in liquid and were exposed to the test parameters while in distilled water (fig. 1). Although viability and phenotype cell counts remained in proportion for the different cuvette environments, number changes occurred. These changes are identified in the discussion of each flight fungal species.

Chaetomium globosum

Survival of the flight organisms remained within one-tenth of a logarithmic concentration of the original inoculum for all test parameters and cuvette types. Viability was reduced in type A cuvettes, and phenotype counts were the highest in the type B cuvettes at full-light exposure (fig. 2). Phenotype numbers and viability rates were stable in vibration controls, flight controls, and ground controls of wet cuvettes. Dry cuvettes in full light yielded an increase in phenotypes, but the viability remained high. Phenotype numbers in dry-cuvette controls were changed insignificantly, and the return viable-cell count remained high.

Rhodotorula rubra

The flight inoculum for type A, type B, and type C cuvettes for all selected test parameters remained within two-tenths of the same logarithmic cell concentration. Full-light exposures produced a noted viability decrease; thus, fewer phenotypes were found. The greatest phenotype return was recorded for the 300-nanometer wavelength in the type A cuvettes; the least return was for the 280-nanometer-wavelength wet cuvettes (fig. 3). Viability remained within the same logarithmic level for test and control wet cuvettes except for those cuvettes that were exposed to full light. The normal rate of phenotype return was present in the control cuvettes. Dry cuvettes produced a greater variation of data compared with the data for wet cuvettes, particularly in the phenotype count per cuvette. Full-light exposures reduced viability, but differences in viability rates between full light and selected wavelengths and between wet and dry cuvettes were less significant for R. rubra than for other flight fungi. Phenotype counts were higher in dry cuvettes that were exposed to a wavelength of 300 nanometers than in those exposed to other wavelengths. Cell return was reduced by a logarithmic level or more in the type B flight control cuvettes compared with type C flight control cuvettes. However, the phenotype count was much higher in both flight (dark) and ground control cuvettes of type C.

Trichophyton terrestre

The flight inoculum for types B and C cuvettes for all controls and test parameters remained within the same two-tenths of one logarithmic level. The cell concentration was higher in the wet cuvettes than in the dry cuvettes. Live-cell returns from type A cuvettes exposed to full light were reduced, and were lower at a wavelength of 280 nanometers compared with 254- and 300-nanometer wavelengths. Phenotype counts were higher in cuvettes exposed to 280-nanometer-wavelength light. All control return cell counts of type A cuvettes remained within the same logarithmic level of viability. Phenotype counts in all type A control cuvettes remained within the normal return as expected for cell populations not exposed to isolated test parameters. Viable-cell return from types B and C cuvettes was reduced most in full-light-exposure cuvettes and was very noticeably reduced at the 280-nanometer wavelength compared with the 254- and 300-nanometer wavelengths. Phenotype count was highest for the 300-nanometer-wavelength cuvette (greatest energy level) (fig. 4). Based on the results of tests of other fungal species and of T. terrestre, the high phenotype count should coincide with high-viability cell returns and should be in opposition to low viability. Thus, cells killed because of exposure to space flight parameters also are variations of phenotype counts. Variation in phenotype counts caused death to the involved cells. All type B control cuvettes returned a viable-cell count within six-tenths of one logarithmic level, and all type C control cuvettes had a return cell count with a one to two logarithmic difference. Cell counts of type B cuvettes were one logarithmic level or more lower than counts from type C cuvettes, whereas phenotype counts were higher in type C cuvettes than in type B cuvettes.

Saccharomyces cerevisiae

The flight control inoculum remained within one logarithmic count for types A, B, and C cuvettes and for the respective parameter deployment. Return viability rates were reduced in cuvettes exposed to 254-nanometer-wavelength light compared with cuvettes exposed to 280- and 300-nanometer-wavelength light. Phenotype counts decreased at energy levels greater than 10^{-3} joule/cuvette (10^{4} ergs/cuvette) at 254- and 280-nanometer wavelengths, but continued to increase at the 300-nanometer wavelength (fig. 5). Cell exposure to dry cuvettes produced no noted change in the rate of viable-cell returns among the three selected wavelengths, but a viability reduction occurred as the exposure increased in energy levels at each respective wavelength. Phenotype counts in dry cuvettes produced the same variation that was noted for the wet cuvettes. Phenotype return was somewhat higher in type C ground, vibration, and flight control cuvettes compared to type B cuvettes.

DISCUSSION

Selected flight phenotype isolates that were subjected to postflight experiments are being studied in an attempt to determine cellular changes incurred during the Apollo 16 flight (ref. 4). Two separate evaluations are underway for nutritional studies of phenotypes. A study of growth rates and changes of fungal strains grown on agar containing various carbon and nitrogen sources and a study on the use of minimal media for analysis of vitamin and amino acid requirements will be performed on some flight fungi.

Results of keratinophilic evaluations of T. terrestre control and phenotypes have indicated dynamic changes in growth rates, sporulation, and mycelial density when human hair from a single source was used as the only available nutrient for in vitro colony development. Light-microscopy studies of filamentous phenotypes will be used to identify changes in growth dynamics, growth rates, and morphology.

The disk method was selected to test several antifungal drugs. Drug-sensitivity variations in flight phenotypes were measured, and flight parameters that caused the maximum changes were identified. Preflight tests were conducted to separate the test drugs into groups based on the greatest growth inhibition with the four flight fungal species.

Mice were injected intraperitoneally with the four flight fungi and with selected phenotypes. The mice were pretreated with cortisone acetate or 5-fluorouracil. Organisms were reisolated from the spleen, the kidney, the liver, and subcutaneous sites near the injection area. Histopathological evaluations of some isolate-induced lesions are being conducted.

The meiotic and mitotic configurations of C. globosum and T. terrestre wild types have been examined and evaluated. Diverse morphological flight phenotypes will be studied for possible configuration variations. C. globosum is capable of decomposing cellulose and other high-molecular-weight carbon compounds. Cotton fabrics and synthetic materials treated with organic compounds can be degraded by the fungus. Scanning electron microscopy will be used to study growth support of C. globosum on fabrics that were used in the Apollo extravehicular-activity suit. Additional studies on phenotypes of the four flight fungi may be performed, depending on the results of current investigations, which include genome-size studies of the flight fungi.

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TABLE I.- DIVERSIFICATION OF THE 23 FUNGI EVALUATED FOR INCLUSION IN FLIGHT EXPERIMENT

Deuteromycetes	Ascomycetes	Oomycetes	Basidiomycetes
Alternaria solani	Chaetomium globosum	Achlya flagellata	Schizophyllum commune
Aspergillus amstelodami	Dipodascus uninucleatus	Saprolegnia parasitica	
Aspergillus ochraceus	Saccharomyces cerevisiae		
Aspergillus niger	Saccharomyces ellipsoideus		
Auerobasidium pullulans	Schizosaccharomyces octosporus		
Cephaliophora tropica			
Nigrospora sphaerica			
Penicillium chrysogenum			
Penicillium italicum			
Penicillium notatum			
Penicillium purpurogenum var. rubri-sclerotium			
Rhodotorula glutinis			
Rhodotorula rubra			
Trichophyton terrestre			

TABLE II.- EXPERIMENTAL ENVIRONMENT OF SACCHAROMYCES CEREVISIAE, TRICHOPHYTON TERRESTRE, AND RHODOTORULA RUBRA IN THE MICROBIAL ECOLOGY EVALUATION DEVICE (MEED)

Expected energy		Species exposed		
Joule/cuvette	Ergs/cuvette	S. cerevisiae	T. terrestre	R. rubra
Exposure peak, 254-nm wavelength				
1×10^{-3}	(1×10^{4})	[a]1, [b]2, [c]3	1, 2, 3	1, 2, 3
2×10^{-3}	(2×10^{4})	1	1	1
4×10^{-3}	(4×10^{4})	1, 2	1, 2	1, 2
5×10^{-3}	(5×10^{4})	3	3	3
Exposure peak, 280-nm wavelength				
9×10^{-4}	(9×10^{3})	2	None	None
1×10^{-3}	(1×10^{4})	1	1	1
2×10^{-3}	(2×10^{4})	1, 2	2, 3	1, 2
5×10^{-3}	(5×10^{4})	1, 2	1	1
7×10^{-3}	(7×10^{4})	1, 3	1, 3	1, 3
9×10^{-3}	(9×10^{4})	1, 2, 3	1, 2, 3	1, 2, 3
2×10^{-2}	(2×10^{5})	3	2, 3	2, 3
Exposure peak, 300-nm wavelength				
8×10^{-4}	(8×10^{3})	1, 2, 3	1, 2, 3	1, 2, 3
2×10^{-3}	(2×10^{4})	1	1	1
4×10^{-3}	(4×10^{4})	1, 2, 3	1, 2, 3	1, 2, 3
5×10^{-3}	(5×10^{4})	1	1	1
7×10^{-3}	(7×10^{4})	1, 2, 3	1, 2, 3	1, 2, 3

[a]Wet (type A) cuvette.

[b]Unvented, dry (type B) cuvette.

[c]Vented, dry (type C) cuvette.

TABLE III.- FULL-LIGHT-EXPOSURE ENVIRONMENT OF CHAETOMIUM GLOBOSUM, TRICHOPHYTON TERRESTRE, AND RHODOTORULA RUBRA IN THE MEED

Expected energy		Species exposed		
Joule/cuvette	Ergs/cuvette	C. globosum	T. terrestre	R. rubra
2×10^{-2}	(2×10^{5})	[a]1, [b]2, [c]3	1, 2, 3	1, 2, 3
2×10^{-1}	(2×10^{6})	1	1	1
2×10^{0}	(2×10^{7})	1, 2, 3	1, 2, 3	1, 2, 3
3×10^{0}	(3×10^{7})	1	1	1
1×10^{1}	(1×10^{8})	1, 2, 3	1, 2, 3	1, 2, 3

[a]Wet (type A) cuvette.

[b]Unvented, dry (type B) cuvette.

[c]Vented, dry (type C) cuvette.

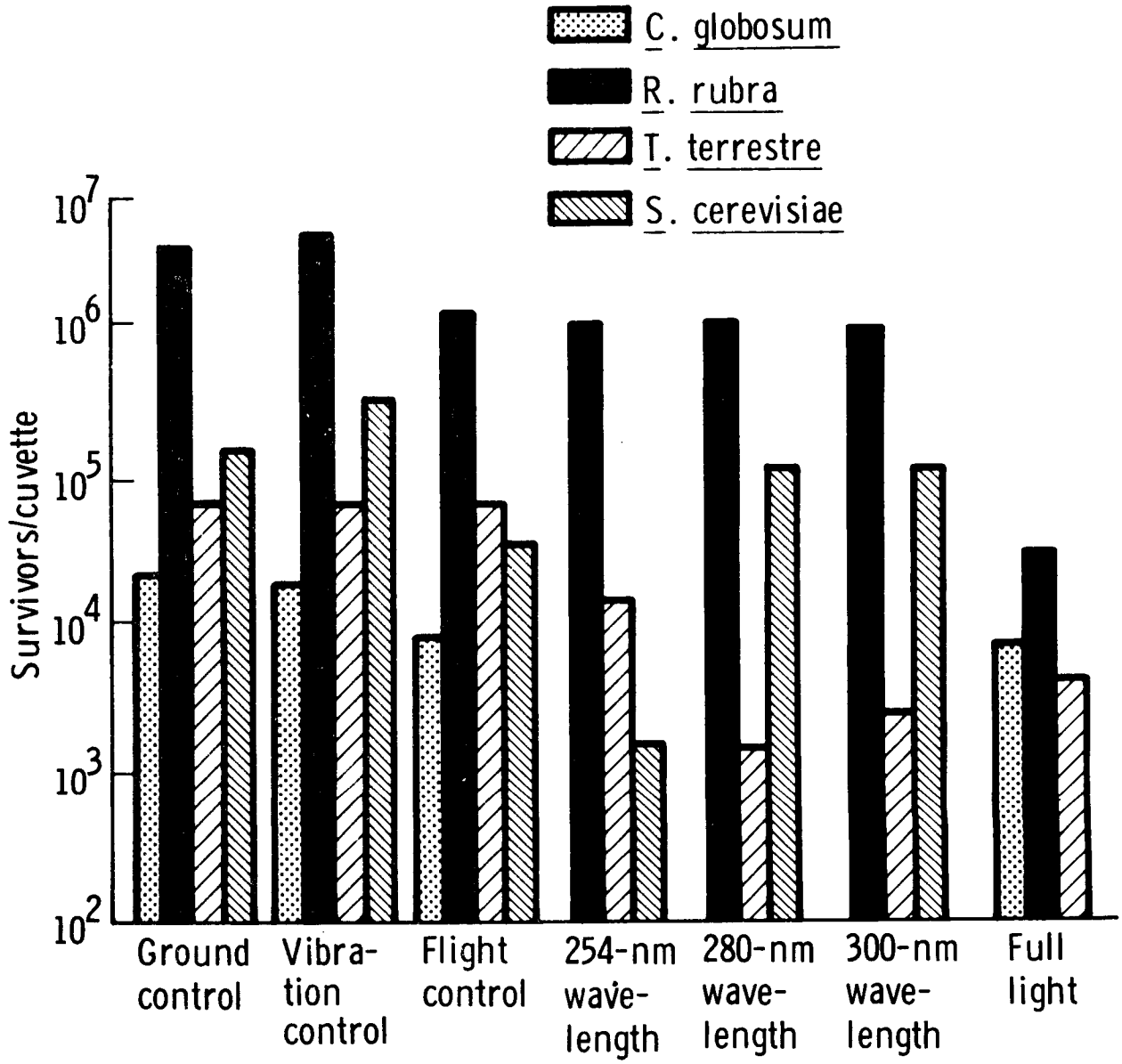

Figure 1.- Survival of fungal cells stored in liquid in cuvettes with each parameter at the lowest energy level.

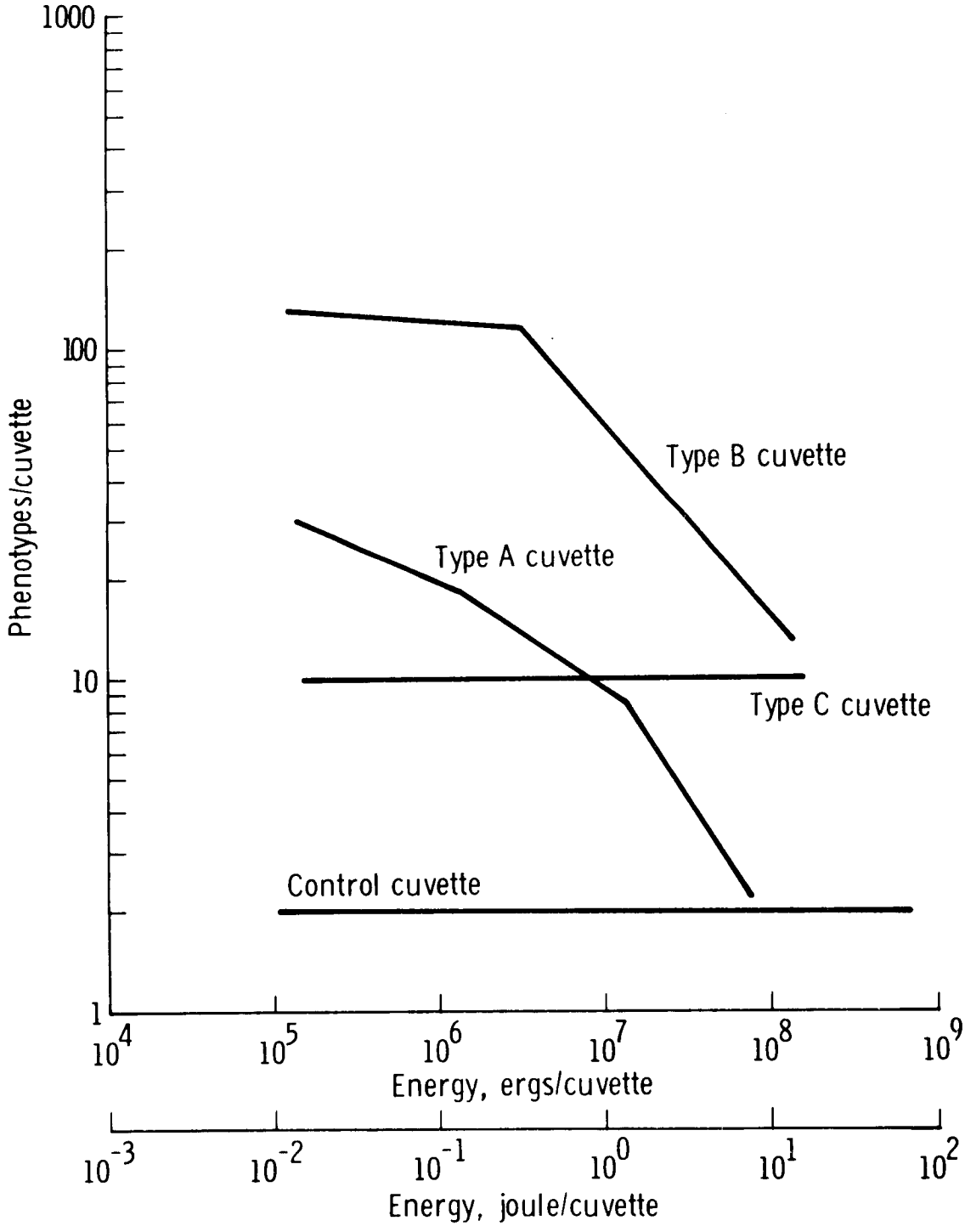

Figure 2.- Phenotype return of C. globosum exposed to full light.

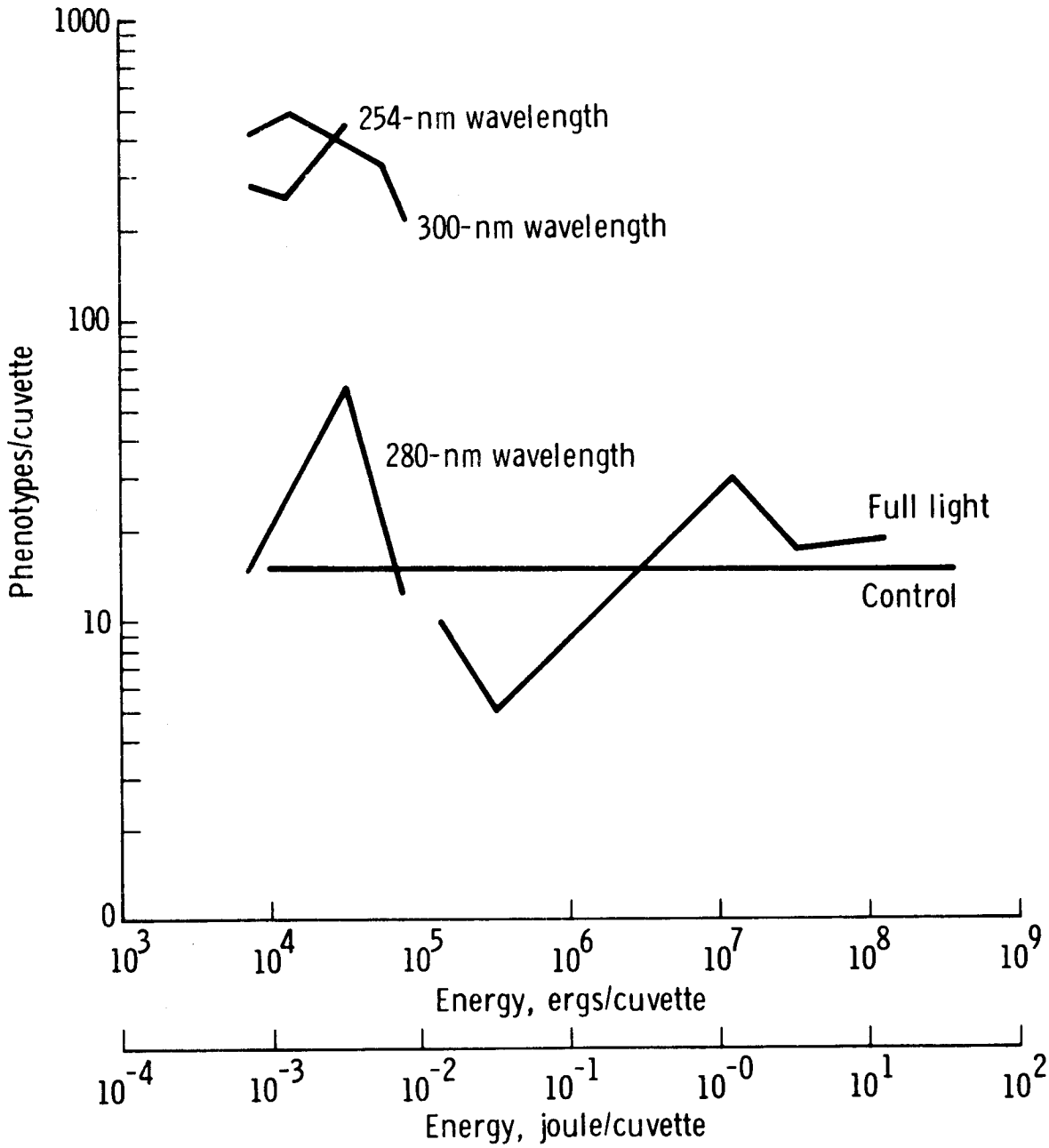

Figure 3.- Phenotype return of R. rubra housed in type A cuvettes.

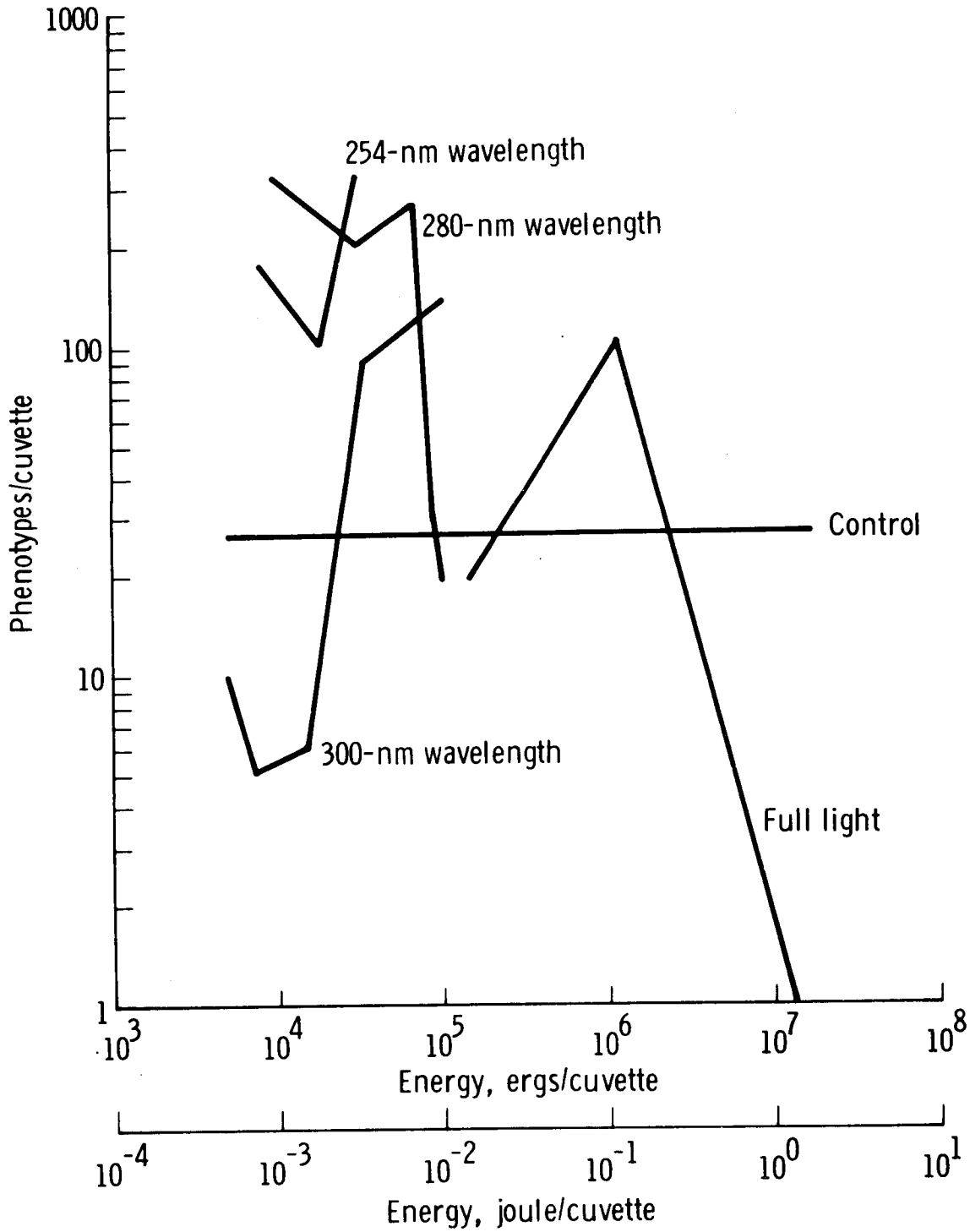

Figure 4.- Phenotype return of T. terrestre housed in type A cuvettes.

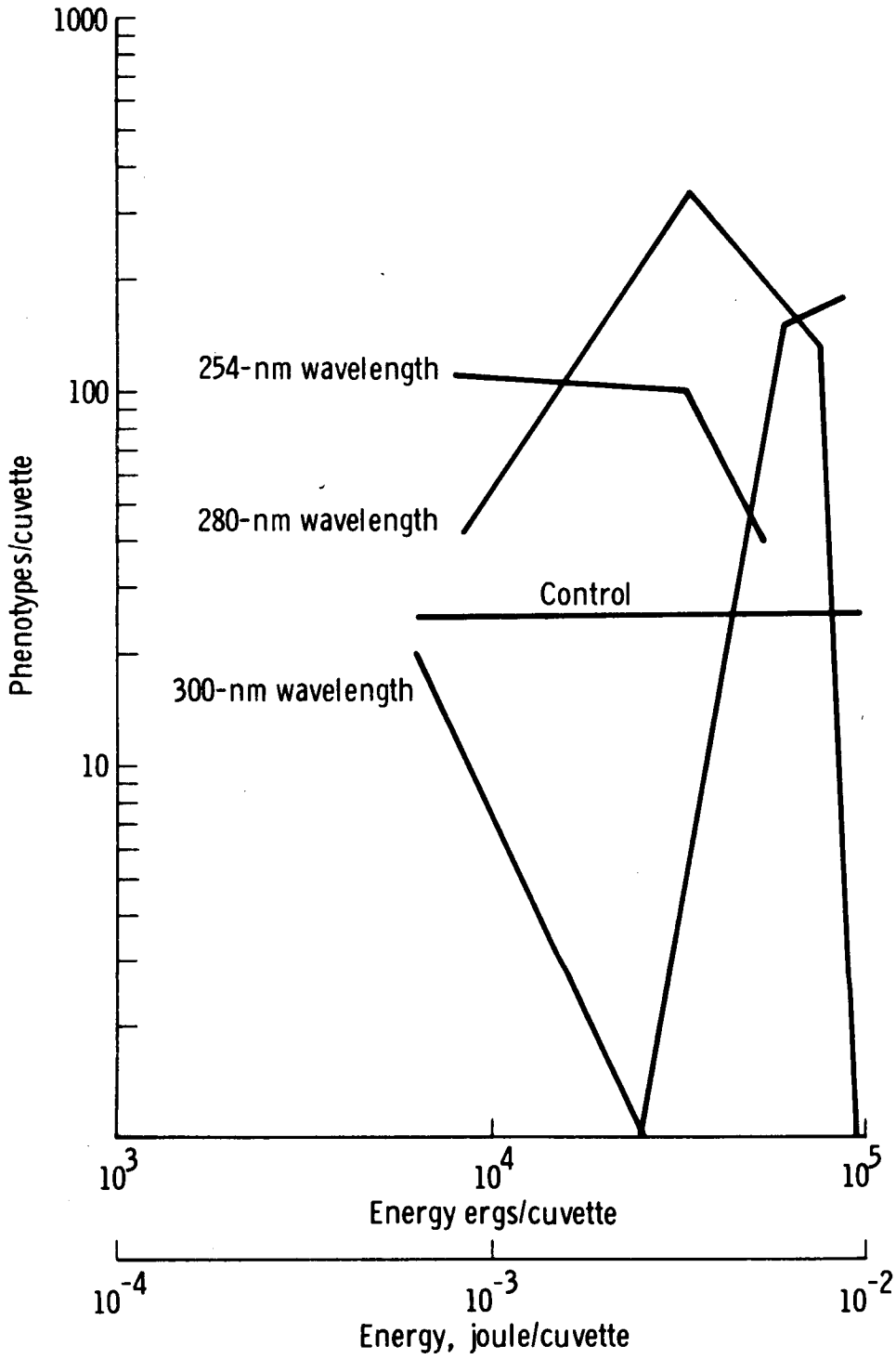

Figure 5.- Phenotype return of S. cerevisiae housed in type A cuvettes.

EFFECT OF SOLAR IRRADIATION ON EXTRACELLULAR ENZYMES OF AEROMONAS PROTEOLYTICA

By Bill G. Foster*

ABSTRACT

The bacterium Aeromonas proteolytica was selected for studying the effects of solar irradiation on extracellular enzymes because it produces an endopeptidase that is capable of degrading proteins and a hemolysin that is active in lysing human erythrocytes. Possible alterations in the rate of enzyme production in response to the test conditions are currently underway and are not available for this preliminary report. Completed viability studies are indicative that little difference exists among the survival curves derived for cells exposed to various components of ultraviolet irradiation in space.

SUMMARY

Aeromonas proteolytica was used to evaluate potential changes in enzyme activity caused by solar irradiation. Aeromonas proteolytica produces a hemolytic factor and an extracellular endopeptidase that elicits intracutaneous hemorrhage and necrosis in laboratory animals. Both the hemolytic factor and the endopeptidase exist in quantitative amounts in crude growth filtrates. Preflight studies established lethal and mutagenic response norms for the microorganism at varied energy levels and two different peak wavelengths of ultraviolet light. These preflight norms were used to establish inflight experimental parameters and to furnish data for postflight comparisons with flight data.

Preliminary results of the postflight studies are indicative that little difference exists in the survival curves among the three wavelengths of irradiation and full light. Assays in enzyme and hemolysin are continuing.

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INTRODUCTION

The possibility that spacecraft environments may select strains of bacteria that are more proteolytic or that produce genetic alterations which result in increased proteolysis was shown in previous studies conducted at Texas A&M University, College Station. On the basis of these findings, it was considered important to include in the Microbial Response to Space Environment Experiment (M191) a bacterial species with easily quantitated extracellular enzymes having some overt biological activity. Aeromonas proteolytica was chosen because one of its extracellular enzymes could be measured accurately and conveniently by use of a urea-denatured-hemoglobin substrate. This endopeptidase, produced in exceptionally high levels, can induce intra-cutaneous hemorrhage and necrosis in laboratory animals. In addition, a hemolysin is found in growth filtrate that is active in lysing human erythrocytes (ref. 1).

While this species is not a part of the endogenous flora of man, it does epitomize a group of microorganisms that may be present on human tissue and that, under stress conditions, could pose medical problems of unknown magnitude. If the hemolytic factor or extracellular endopeptidase, or both, produced in small amounts by many endogenous microbes, were to be produced in unusually high quantities or altered so as to be more effective, a serious health problem might exist for space travelers.

Preflight studies necessarily included such problems as the following.

(1) Development of a nonnutritive or low-nutritive holding medium

(2) Establishment of baseline information for the standardization of the assay for endopeptidase levels and hemolytic titers

(3) Development of a method by which intracutaneous hemorrhage could be quantitated in guinea pig tissue

(4) Evaluation of the response of these microorganisms to parameters of space flight and experimentation

METHODS AND RESULTS OF PREFLIGHT CALIBRATIONS

Microorganism Used

The strain of Aeromonas proteolytica used in this study was a subculture of the original isolate of Merkel and Traganza (ref. 2). The subculture was obtained from Dr. J. M. Prescott, Department of Biochemistry-Biophysics, Texas A&M University, and was maintained on tryptic soy agar.

Endopeptidase Assay

The endopeptidase activity was measured by using a urea-denatured-hemoglobin substrate. For the assay, 1.0 cm^3 (1.0 milliliter) of a 1:40 dilution of the cultural filtrate containing the enzyme was incubated with 5.0 cm^3 (5.0 milliliters) of the hemoglobin substrate for 5 minutes in a 310° K (37° C) water bath. At the end of this period, 10 cm^3 of 5% (weight/volume) trichloroacetic acid in water was added to terminate the enzyme action and to precipitate the undigested protein, which was removed by filtration. The absorbance of the filtrate and control was read at a wavelength of 280 nanometers on a spectrophotometer, and the activity was expressed in units. One unit of endopeptidase activity is defined as the amount of enzyme producing an absorbance of 1.0 in 5 minutes at 310° K (37° C) under the assay conditions described.

Hemolysin Assay

The degree of hemolysis produced by the cultural filtrate was determined on 2-percent human type O, Rhesus (Rh) positive erythrocytes, diluted in physiological saline. Doubling dilutions to 1:1024 were made of the filtrate in physiological saline with a final concentration of 0.2-percent sodium azide added to prevent contaminating growth. A modified microtiter method was used. Five drops of the culture filtrate and three drops of the 2.0-percent human-erythrocyte suspension was dispensed in wells of a Microtest II tissue culture plate. The hemolysin titer is defined as the reciprocal of the highest dilution that gives complete clearing in the well following 24 hours of incubation at 310° K (37° C).

Hemorrhagic and Necrotic Activity

Crude growth filtrate, when inoculated into guinea pigs, produced immediate erythema followed within 5 minutes by intracutaneous hemorrhage and, in some cases, necrosis. Unless severe, the lesions scabbed within 24 hours. The degree of reaction was evaluated by measuring the extension of the scabbing at its greatest distance (ref. 3). By diluting the filtrate, areas of reaction were controlled so that a number of tests could be made on the flank of one animal.

Development of Holding Solution

Several nonnutritive or low-nutritive holding solutions were studied, including distilled water, deionized distilled water, artificial sea water, physiological saline, physiological saline buffered with

dibasic potassium phosphate, and 1 percent asparagine in distilled water. Several studies were made by holding a predetermined concentration of microorganisms in each test solution for 20 days at 297° K (20° C). Periodic viability checks were made, and the percentage of survival was calculated. In preliminary tests, holding solution aliquots of 5.0 cm^3 (5.0 milliliters) were used. After selection of physiological saline buffered with 0.5 kg potassium phosphate/m^3 (0.5 g K_2HPO_4/liter) as the holding solution, numerous tests were made in 0.05-cm^3 (50 microliter) quantities. The survival consistently ranged from 22 to 33 percent.

Preflight Ultraviolet Irradiation and Mutation Study

A 140-watt, 115-volt, utility broad-spectrum quartz lamp was used for the ultraviolet (uv) light studies. Bandpass filters that transmitted light at a peak wavelength of either 254 or 280 nanometers were used. To ensure accurate transmittance of the proper energies to the samples, the light source was calibrated with these filters by using the potassium ferrioxalate actinometry procedure of Hatchard and Parker (ref. 4).

In preparation for the irradiation, an inoculum of A. proteolytica was transfered into 100 cm^3 (100 milliliters) of 2-percent casitone in artificial sea water (27 kg/m^3 (27 g/liter) of Seven Seas Marine mix). Growth was at room temperature for 18 hours on a low-speed magnetic stirrer. Ten cm^3 (10 milliliters) of this primary culture were transferred to another flask containing 100 cm^3 (100 milliliters) of 2-percent casitone in sea water and were allowed to grow for 24 hours under previous conditions. Cells from this secondary culture were sedimented at 8000 rpm for 15 minutes in a refrigerated centrifuge at 277° K (4° C). The cells then were washed three times by centrifugation in physiological saline containing 0.05 kg K_2HPO_4/m^3 (0.05 g K_2HPO_4/liter). The concentration of this suspension was adjusted to give a reading of 0.1 optical density (OD) at a wavelength of 660 nanometers on a spectrophotometer. When diluted 1:50, the suspension yielded a viable-cell count of approximately 10^6 organisms/cm^3 (10^6 organisms/ml) and an OD of approximately 0.02.

The dilute suspensions were irradiated in 0.05-cm^3 (50 microliter) capacity cuvettes, constructed of black plastic to avoid reflection. Each cuvette possessed a single 25-mm^2 quartz window, designed to provide unrestricted passage of uv light and a flat field of irradiation.

The light source was fastened in a special stand to maintain a 30-centimeter distance between the sample and the light source and to ensure a constant perpendicular angle of irradiation. By using this stand, variations in degree of reflection and absorption were eliminated. The cuvettes containing the samples were placed under the light source in triplicate for the predetermined time calculated to give an exposure of the desired intensity. The energy levels used at both wavelengths were 1×10^{-5}, 5×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3}, 1×10^{-2}, and 5×10^{-2} joule/cuvette (1×10^{2}, 5×10^{2}, 1×10^{3}, 5×10^{3}, 1×10^{4}, 5×10^{4}, 1×10^{5}, and 5×10^{5} ergs/cuvette).

Immediately following each irradiation, 100-fold dilutions of the samples were made through 10^{5} in artificial sea water. Then, spread plates were made in duplicate on heart infusion agar from each of the dilutions. Fifty clones of survivors from each energy level of each wavelength were selected and transferred to heart infusion agar slants. As a control, three cuvettes were loaded and unloaded in the same manner as the irradiated cuvettes, but without exposure to light. Because the irradiations with the two wavelengths were completed at separate times, by use of separate primary cultures from different stock slants, a separate control was included for each wavelength.

The dose-response curves of A. proteolytica to irradiations at peak wavelengths of 254 and 280 nanometers follow the same general trend, as shown in figures 1 and 2. However, the energy level at which a noticeable effect on survival first occurred was slightly lower for the 280-nanometer-wavelength group than for the 254-nanometer-wavelength group. A sharp, steady decline of survivors began above 10^{-4} joule/cuvette (10^{3} ergs/cuvette) of irradiation at a wavelength of 254 nanometers and terminated with a total kill at 5×10^{-2} joule/cuvette (5×10^{5} ergs/cuvette) at the same wavelength. The same sharp decline occurred for the group irradiated at a wavelength of 280 nanometers. In this case, the decline began above 5×10^{-5} joule/cuvette (5×10^{2} ergs/cuvette) with a total killing at 1×10^{-2} joule/cuvette (1×10^{5} ergs/cuvette).

Postirradiation Evaluation

The culture filtrates of A. proteolytica were prepared for the postirradiation evaluation as follows. In a 250-cm^3 (250 milliliter) flask, 50 cm^3 (50 milliliters) of 2-percent casitone in artificial sea water were heavily inoculated with growth from a heart infusion agar slant and

incubated on a reciprocal shaker for 28 hours at room temperature. Pilot studies were indicative that these were the optimum conditions for maximum enzyme production. The cells were removed by centrifugation at 8000 rpm for 10 minutes in a refrigerated centrifuge at 277° K (4° C). The cell-free culture filtrate was analyzed for endopeptidase activity, hemolysin titer, and, in some cases, guinea pig necrosis.

A distribution of 99-percent confidence was established with the control group by analysis of the endopeptidase production. Experimental clones producing endopeptidase activity outside the 99-percent confidence range were regrown and reassayed four times to ascertain stability of the deviation. Eleven clones of the organisms exposed to irradiation at a wavelength of 254 nanometers were found to produce endopeptidase activity lower than this 99-percent limit, but none higher. Of the samples exposed to irradiation at a wavelength of 280 nanometers, none produced activity lower or higher than the calculated range. The mutation frequency for the group irradiated at a wavelength of 254 nanometers is shown in figure 1.

No great deviation occurred in the hemolysin titers produced by the clones exposed to either of the wavelengths of irradiation. However, there was a slightly different distribution of the percent of clones producing particular titers (table I).

METHODS AND RESULTS OF FLIGHT EXPERIMENT

Preparation of Culture

The culture of A. proteolytica used for this experiment (3-16-72-2) has an endopeptidase activity of 16 units as determined by the urea-denatured-hemoglobin-substrate assay for endopeptidase.

A starter culture of A. proteolytica was prepared by inoculation from a stock slant (trypticase soy agar) into 100 cm^3 (100 milliliters) of 2-percent casitone in artificial sea water. The culture was grown for 18 hours at room temperature on a low-speed magnetic stirrer, and 1 cm^3 (1 milliliter) of the 18-hour broth culture was inoculated into a second 100-cm^3 (100 milliliters) flask containing a 2-percent casitone in artificial sea water. The culture was grown 24 hours under the previously discussed conditions, and 15 cm^3 (15 milliliters) from this culture were sedimented by centrifugation at 8000 rpm (7710 × g) for 20 minutes at 277° K (4° C). The cells subsequently were washed twice with sterile holding solution by centrifugation. The cells from the final wash were suspended in 10 cm^3 (10 milliliters) of holding solution and were used to

prepare cell suspensions adjusted to an OD of 0.1, 0.15, 0.2, 0.25, 0.3, 0.43, and 0.5 as recorded on a spectrophotometer at a wavelength of 660 nanometers. Each of the resulting cell suspensions was diluted again to 1:50 and checked on a spectrophotometer to confirm that the percent transmission had not dropped below 90 percent. An aliquot of each dilution was plated on heart infusion pour plates to determine the number of surviving microorganisms. The suspension yielding the largest number of microorganisms while not exceeding the optical limit was used to fill the flight cuvettes.

Postflight Survival

The flight cuvettes were opened after flight, and the content was plated for surviving organisms. Dilutions of 10^3, 10^4, and 10^5 in duplicate were made from each cuvette. After 24 hours, incubation counts were made, and averages were computed.

The survival curves for flight-irradiated test systems are presented in figures 3 to 6. An analysis of the curves is indicative that there is no statistical difference among these survival patterns and the number of deaths resulting from storage. Also, there is no statistical difference in the death response among the three energy levels and full light. These viability rates were not unexpected because of the demonstration in preflight studies of a repair mechanism following the irradiation of starved cells and storage for several days.

Twenty-four hours after plating the content of each cuvette, 50 clones were selected at random from each of the energy levels at the four wavelengths. Each was transferred to heart infusion agar slants. Each clone was inoculated into 50 cm^3 (50 milliliters) of 2-percent casitone broth for cultivation. Following incubation for 28 hours at room temperature with constant aeration by shaking, the culture was sedimented by centrifugation at 8000 rpm for 10 minutes at 277° K (4° C). The resulting supernatant was frozen until assayed. Clones not cultured were transferred to fresh slants after 14 days.

The 10^2 dilution made from each cuvette was retained and stored at 293° K (20° C). Eight weeks after the opening of the cuvette, aliquots of the first dilution (10^2) were checked for viable microorganisms. If found viable, additional dilutions were made, and 30 additional clones from each wavelength and energy level were selected for study and prepared for assay as described. Assays are continuing.

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4. Hatchard, C. G.; and Parker, C. A.: A New Sensitive Chemical Actinometer: I. Potassium Ferrioxalate as a Standard Chemical Actinometer. Proc. Roy. Soc. London, A, vol. 235, 1956, pp. 518-536.

TABLE I.- HEMOLYSIN TITERS

Exposure		Percent of group having the indicated titer				Average titer of group
Joule/cuvette	Ergs/cuvette	16 (a)	32	64	128	
254-nm wavelength group						
Control	Control	2	38	34	26	67.52
1×10^{-2}	1×10^{5}	2	24	56	18	66.88
5×10^{-3}	5×10^{4}	0	13	42	44	88.17
1×10^{-3}	1×10^{4}	0	12	44	44	88.32
5×10^{-4}	5×10^{3}	0	24	44	32	76.80
1×10^{-4}	1×10^{3}	24	44	30	2	39.68
5×10^{-5}	5×10^{2}	0	31	65	4	56.67
1×10^{-5}	1×10^{2}	0	6	31	61	99.55
280-nm wavelength group						
Control	Control	0	6	58	36	85.12
1×10^{-5}	1×10^{2}	0	54	38	8	51.84
5×10^{-5}	5×10^{2}	0	32	66	2	55.04
1×10^{-4}	1×10^{3}	0	44	56	0	44.92
5×10^{-4}	5×10^{3}	0	40	56	4	53.76
1×10^{-3}	1×10^{4}	0	16	82	2	60.16
5×10^{-3}	5×10^{4}	0	42	58	0	50.56

[a]The hemolysin titer is defined as the reciprocal of the highest dilution that exhibits complete clearing of the well after 24-hour incubation at 310° K (37° C), by use of 2-percent human erythrocytes. The titer of each sample represents the higher of two runs.

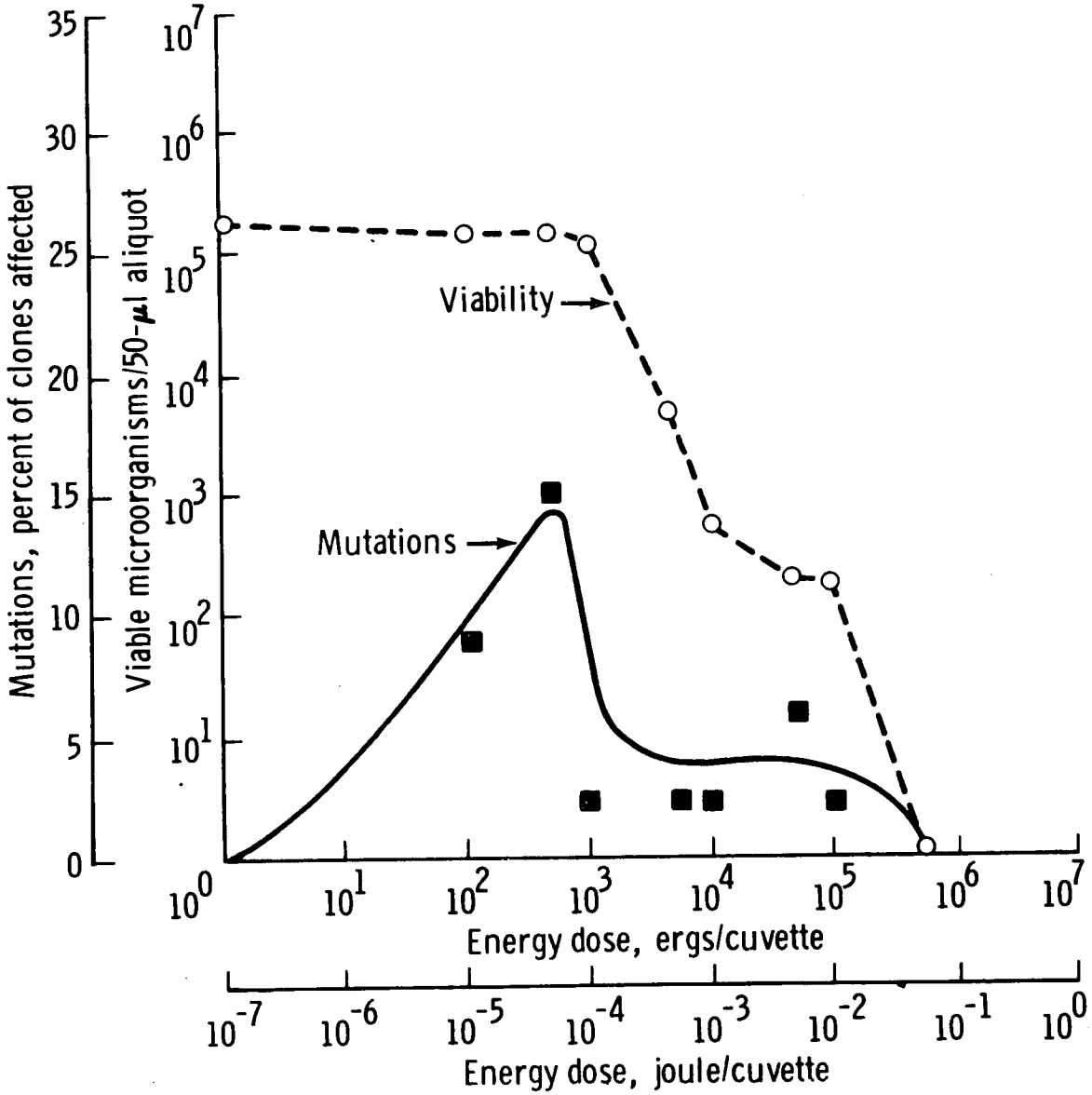

Figure 1.- Ultraviolet dose response and mutation frequency of Aeromonas proteolytica for irradiations at a peak wavelength of 254 nm, preflight data.

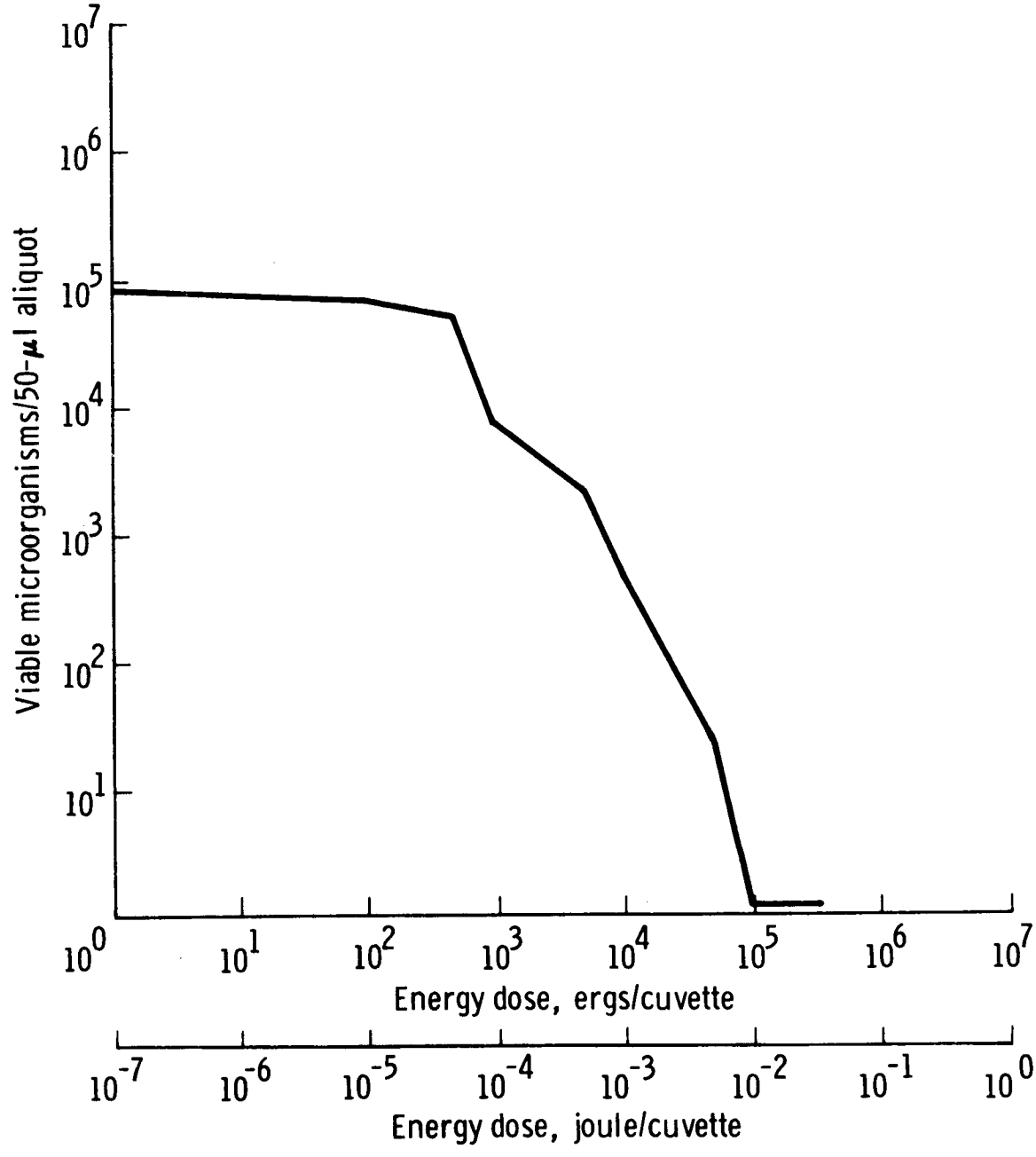

Figure 2.- Ultraviolet dose response of Aeromonas proteolytica for irradiations at a peak wavelength of 280 nm, preflight data.

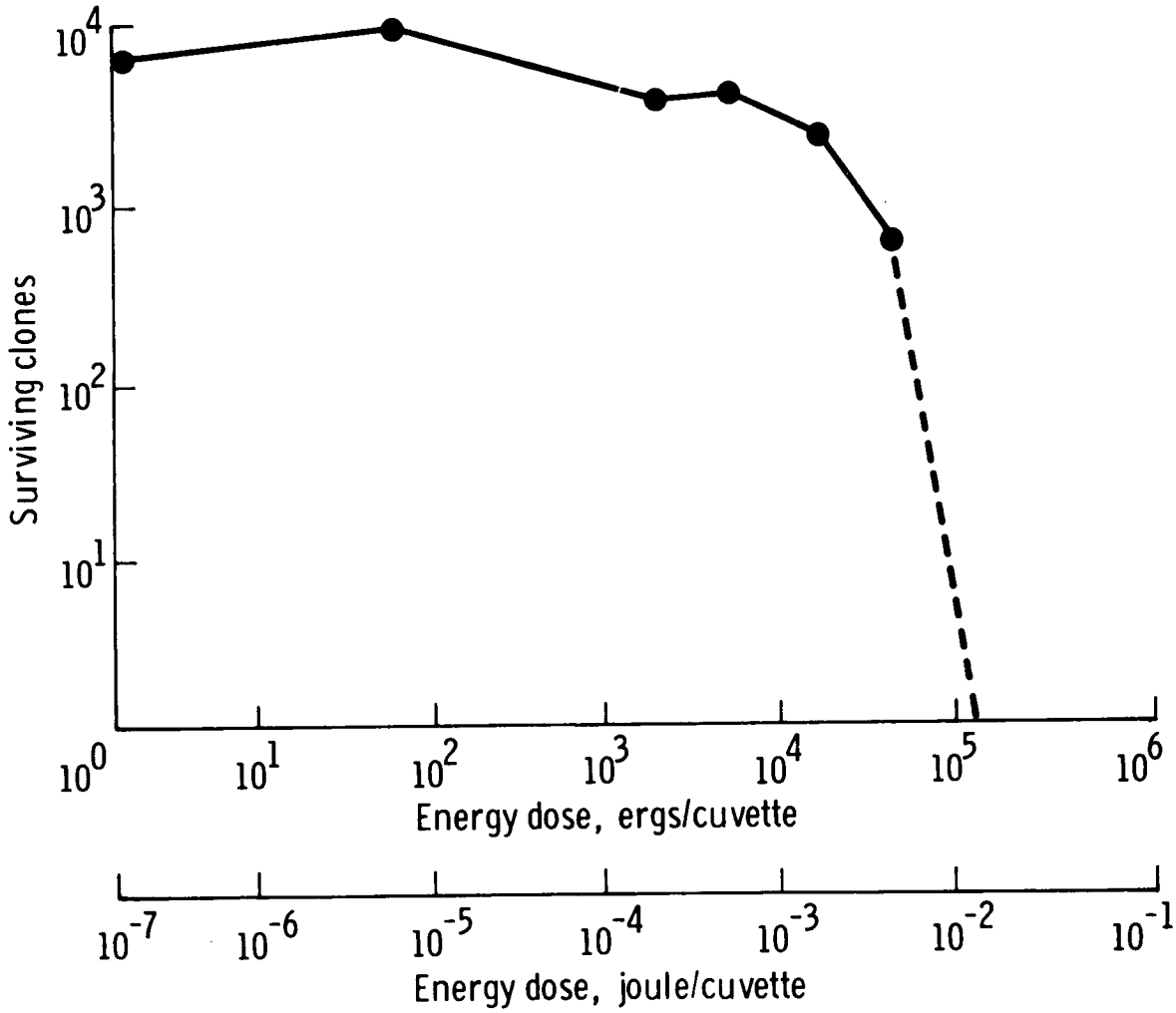

Figure 3.- Effect of solar irradiation on Aeromonas proteolytica at a peak wavelength of 254 nm, flight data.

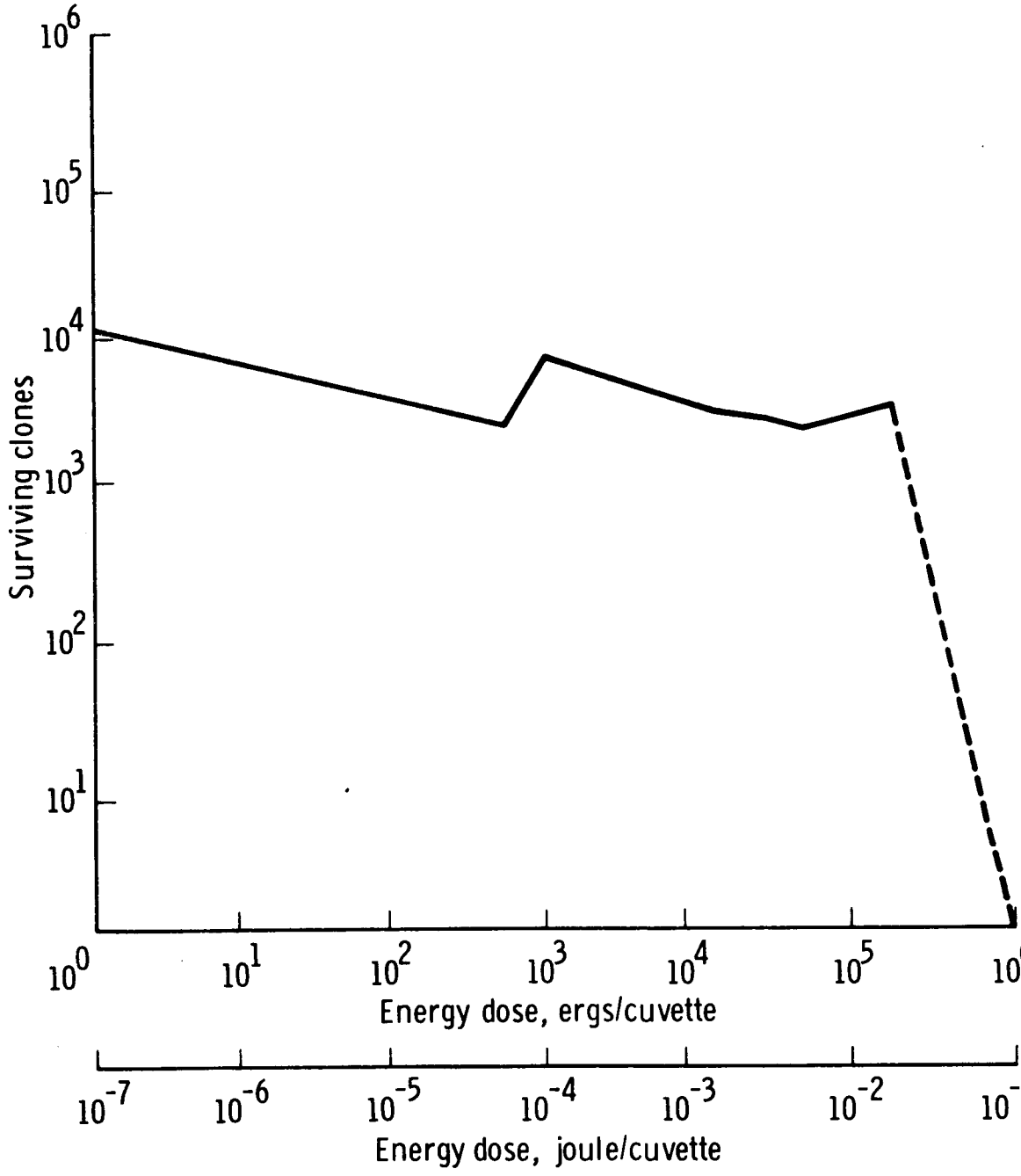

Figure 4.- Effect of solar irradiation on Aeromonas proteolytica at a peak wavelength of 280 nm, flight data.

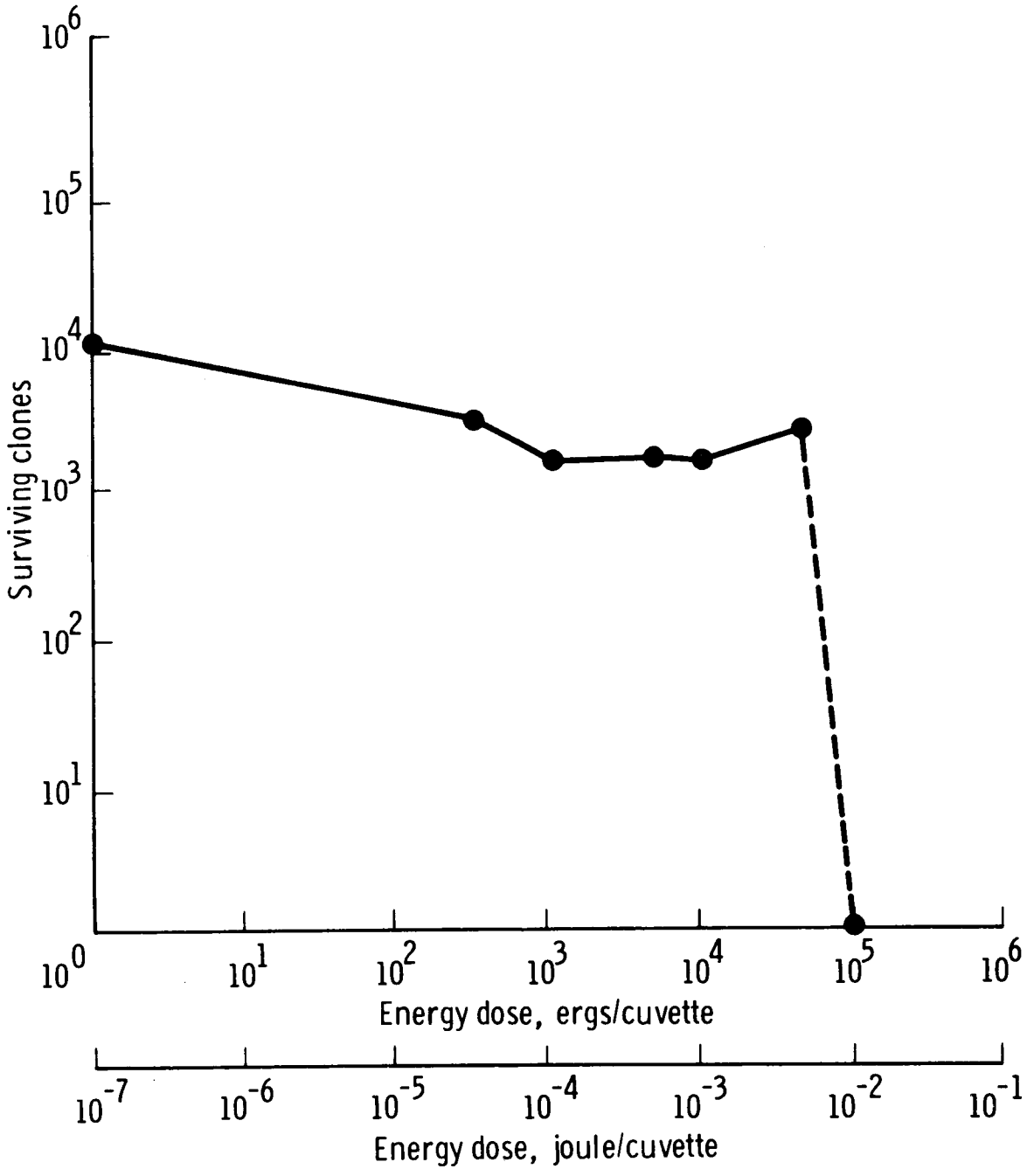

Figure 5.- Effect of solar irradiation on Aeromonas proteolytica at a peak wavelength of 300 nm, flight data.

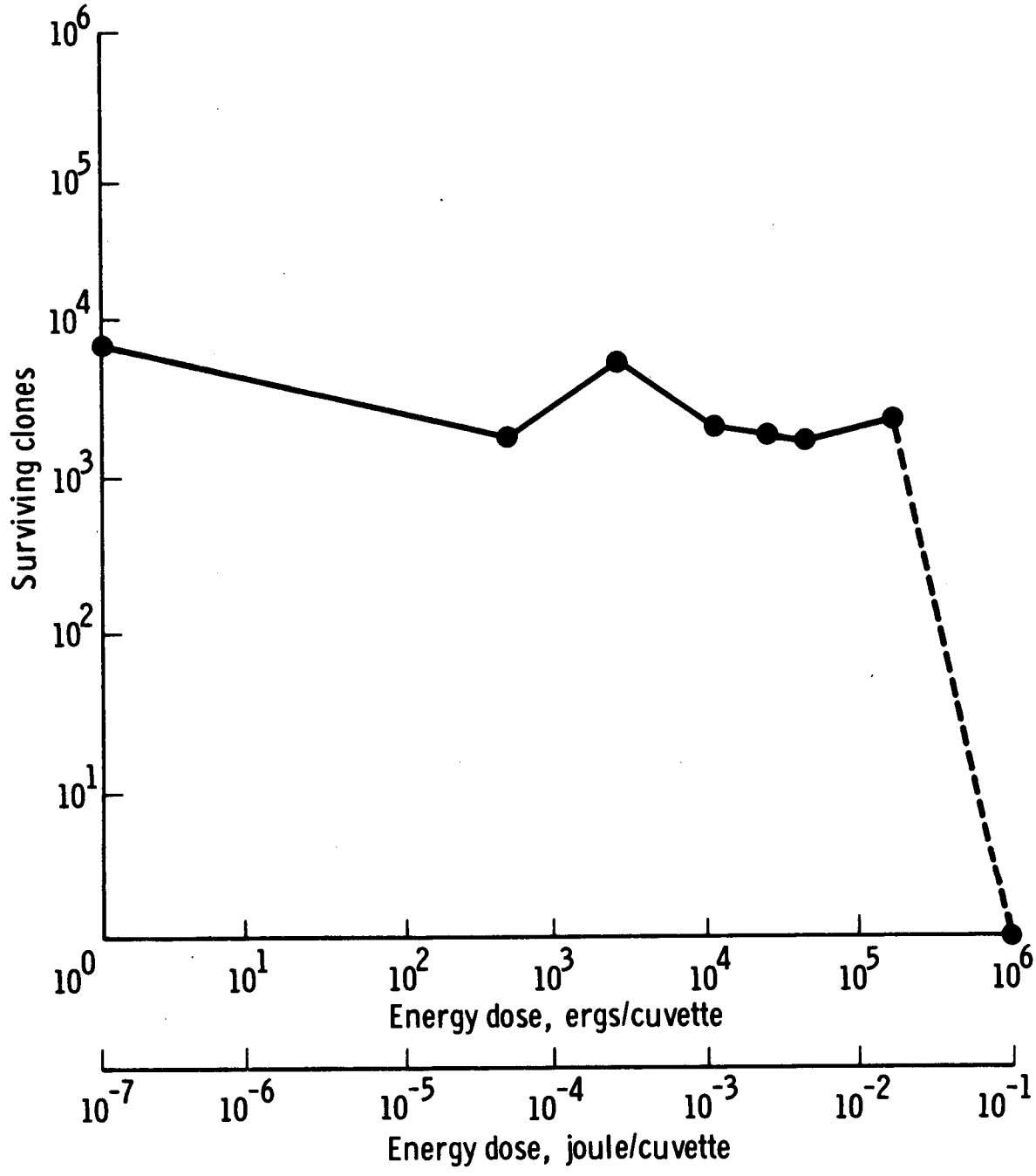

Figure 6.- Effect of solar irradiation on Aeromonas proteolytica with full light, flight data.

SECTION III SUPPORTING PHYSICAL MEASUREMENTS

THE USE OF FILM ACTINOMETRY IN LOW-INTENSITY ULTRAVIOLET IRRADIATION MEASUREMENT

By Richard A. Long*

ABSTRACT

High-resolution film was used to measure solar ultraviolet irradiation at a wavelength of 254 nanometers between energy levels of 3×10^{-7} and 130×10^{-7} joules (3 and 130 ergs). The results imply that the film recorded exposure to energy levels from 2.5 to 13 times the expected values. These high values are being evaluated for the influences of other factors that could affect the recorded values.

SUMMARY

Optical density was calculated based on flight experimental film, resulting in values that exceeded the measurable range of predetermined curves. The high optical-density values are indicative that the flight experimental film was exposed to higher-than-anticipated energy levels. These high values were caused by either errors in the predetermined filter ratings or other unaccountable irradiations in the space environment. Further evaluation of individual filter packs will provide explanations of discrepancies between the calculated and expected energy levels.

INTRODUCTION

Actinometric techniques have been used to determine the amount of radiant energy striking biological systems. This energy, expressed in joules or ergs, is a function of the change in optical density of the medium through which the light energy is transmitted. Photographic film

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is a sensitive detector of radiant energy and provides a permanent record of the radiation level to which the film has been exposed. Then, the optical density of the exposed film may be correlated to predetermined calibration data to obtain the energy level to which selected biological systems have been subjected.

METHODS

High-resolution film was selected for this experiment because of the response to ultraviolet (uv) irradiation at a wavelength of 254 nanometers between energy levels of 10^{-5} to 10^{-4} joule (10^{2} to 10^{3} ergs). Film response to these energy levels was satisfactory for laboratory experiments. However, subsequent storage of the film after exposure and before processing resulted in a rapid loss of the latent image formed on the film. The presence of oxygen and moisture has been implicated as a contributing factor in the image loss within the film emulsion. To minimize image loss and to increase the reliable time interval between exposure and processing, the film was sensitized before exposure (fig. 1). The sensitization process resulted in a linear response to uv irradiation in the energy range of 3×10^{-7} to 1.2×10^{-5} joule (3 to 120 ergs) and a minimum image loss for storage for as many as 5 days after exposure.

All mission film chips were punched (fig. 2) from a single 10.16- by 12.7-centimeter (4 by 5 inch) sheet of film under red-light conditions and loaded into 135 individual dry (type B) cuvettes using a vacuum pick. Next, a black paper spacer and two Lexan gaskets were fitted over the film chip. All partially loaded cuvettes were placed in a 1×10^{-3}-cm^{3} (1 liter) stainless steel bolt-top can (BTC) configured for high-vacuum testing. The can was mounted on a cryogenic-absorption ion vacuum pump and evacuated to a pressure of 1.33×10^{-3} N/m^{2} (1×10^{-5} torr) for 1 hour (fig. 3). The can was backfilled with dry nigrogen (N_2) gas, sealed, and removed from the vacuum unit. This sensitizing procedure rendered the film emulsion free of oxygen and residual water vapor.

An isolator (fig. 4) for maintenance of germ-free animals was modified to accommodate the cuvettes in a dry N_2 atmosphere until the loading process was completed. The sealed BTC was transferred to the pass-through chamber of the isolator and was subjected to continuous N_2 flow for 18 hours. At the end of the wash cycle, the BTC was transferred into the main chamber and opened under red-light conditions. The cuvettes were assembled and sealed (fig. 5), returned to the BTC, and stored in the isolator until ready for loading into the respective Microbial Ecology

Evaluation Device (MEED) trays. Twenty-four film cuvettes were placed under seven neutral-density filters to monitor the solar uv energy transmitted at a peak wavelength of 254 nanometers in the energy range of 1.1×10^{-6} to 1.1×10^{-5} joule (11 to 110 ergs) total energy. The MEED assembly was transported to the NASA John F. Kennedy Space Center and was loaded on board the Apollo 16 command module. Exposure to solar irradiation occurred 9 days after launch, and the cuvettes were received in the NASA Lyndon B. Johnson Space Center Lunar Receiving Laboratory 5 days after splashdown. Film processing was initiated immediately upon receipt.

Film chips were removed individually from each test and control cuvette by the use of a vacuum pick (fig. 6) and were transferred to the unloading plate. The chip was removed from the plate receptacle (fig. 7) by the use of a spring-loaded tweezer clip and was mounted on the agitator carrousel (fig. 8). The fully loaded carrousel was mounted on the motor-driven agitator assembly (fig. 9). One film chip from each control and each experimental group was processed together as a unit to ensure validity of comparison among the groups. Processing was facilitated by the use of a continuous-flow circulator that maintained the developing solutions at a constant temperature of 293° K (20° C). Each processing sequence was completed by transferring the agitator assembly from well to well of the processing tank. The carrousel was removed from the agitator assembly, rinsed in running water for 10 minutes, and dried for 20 minutes at 310° K (37° C). All control and experimental film was processed within 24 hours and was stored in individual prelabeled glassine envelopes until ready for optical-density analysis. Spectrophotometric data were collected from individual film chips and were converted to optical densities by the use of a formula. Correlation of the mean optical densities of the nonirradiated ground, vibration, and flight control groups was within ±1 percent, indicating reliability of all control groups.

RESULTS AND DISCUSSION

Three of the filters monitored were rated to transmit energies in the energy range of 1.1×10^{-6} to 1.9×10^{-6} joule (11 to 19 ergs). Data derived from the returned film chips are suggestive that these filter combinations transmitted energies in excess of 13 times the expected levels. The sensitization of the film emulsion with N_2 gas (which was required to decrease latent-image fading) necessitated the use of these extremely dense filters, although these filters were not used with any of the other test systems. The results are indicative that the filters were less accurate than desirable when used in this particular manner; thus, calculations based on such severely attenuated irradiation generate

a prohibitive level of error. Several factors were contributing to this error that would not significantly affect other parts of the experiment system. Minuscule light leaks, galactic irradiation, variations in filter manufacturing, and optical pressure lines at filter interfaces are the causes of most of the observed inconsistencies when such small quantities of irradiation are used. Detailed testing of each filter pack is required to establish the exact nature of this discrepancy.

Three additional filters, which were of a different type, were rated in the energy range of 7×10^{-6} to 8.7×10^{-6} joule (70 to 87 ergs). From test data, it can be concluded that these filters transmitted energies in excess of 2.5 times the expected energy levels. The discrepancy is considerably less in this higher energy range, and the disturbing effect of minor inconsistencies still can be demonstrated. Film exposed under the remaining filter rated at 1.1×10^{-5} joule (110 ergs) was overexposed, and reliable data could not be collected.

The data points used to plot the flight experiment curve (fig. 10) were gathered as a ground-based experiment and represent the points around which the flight experiment data were anticipated to fall. The energy levels bounded by brackets represent the actual range of flight experimental data based on a hypothetical extension of the curve from 1.29×10^{-5} to 3.11×10^{-5} joule (129 to 311 ergs). The standard curve is included for reference.

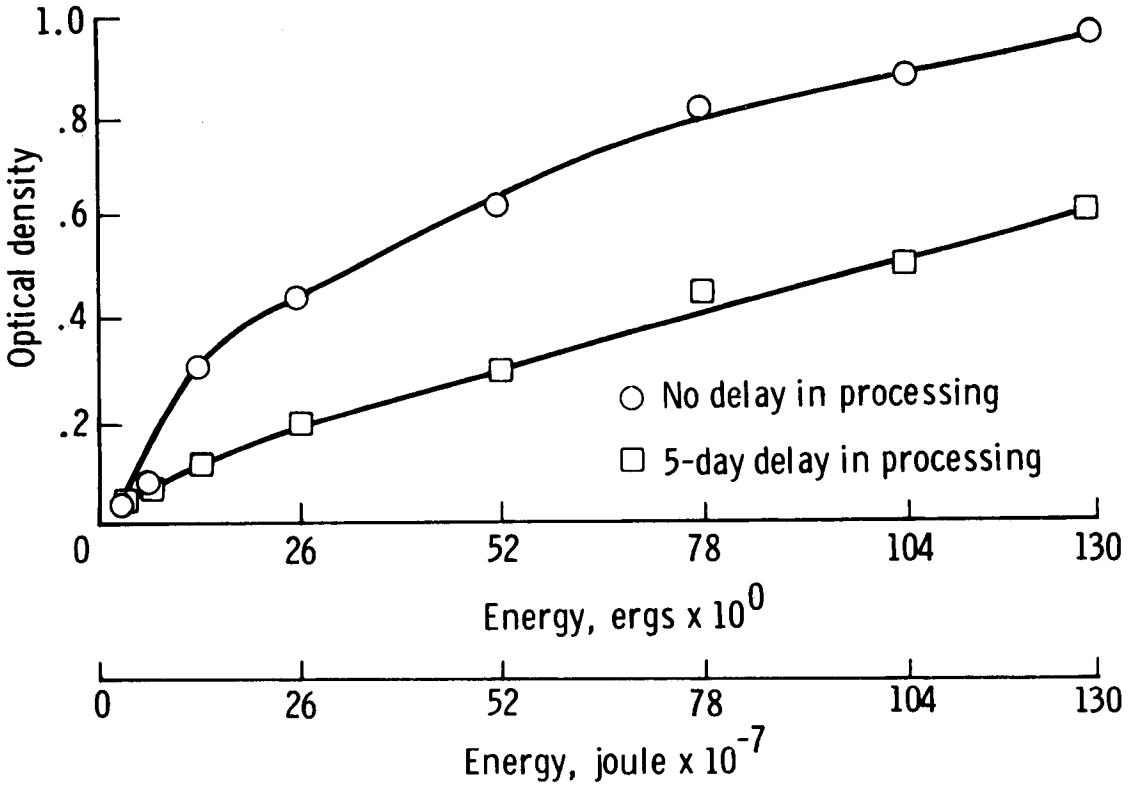

Figure 1.- Sensitivity of high-resolution film.

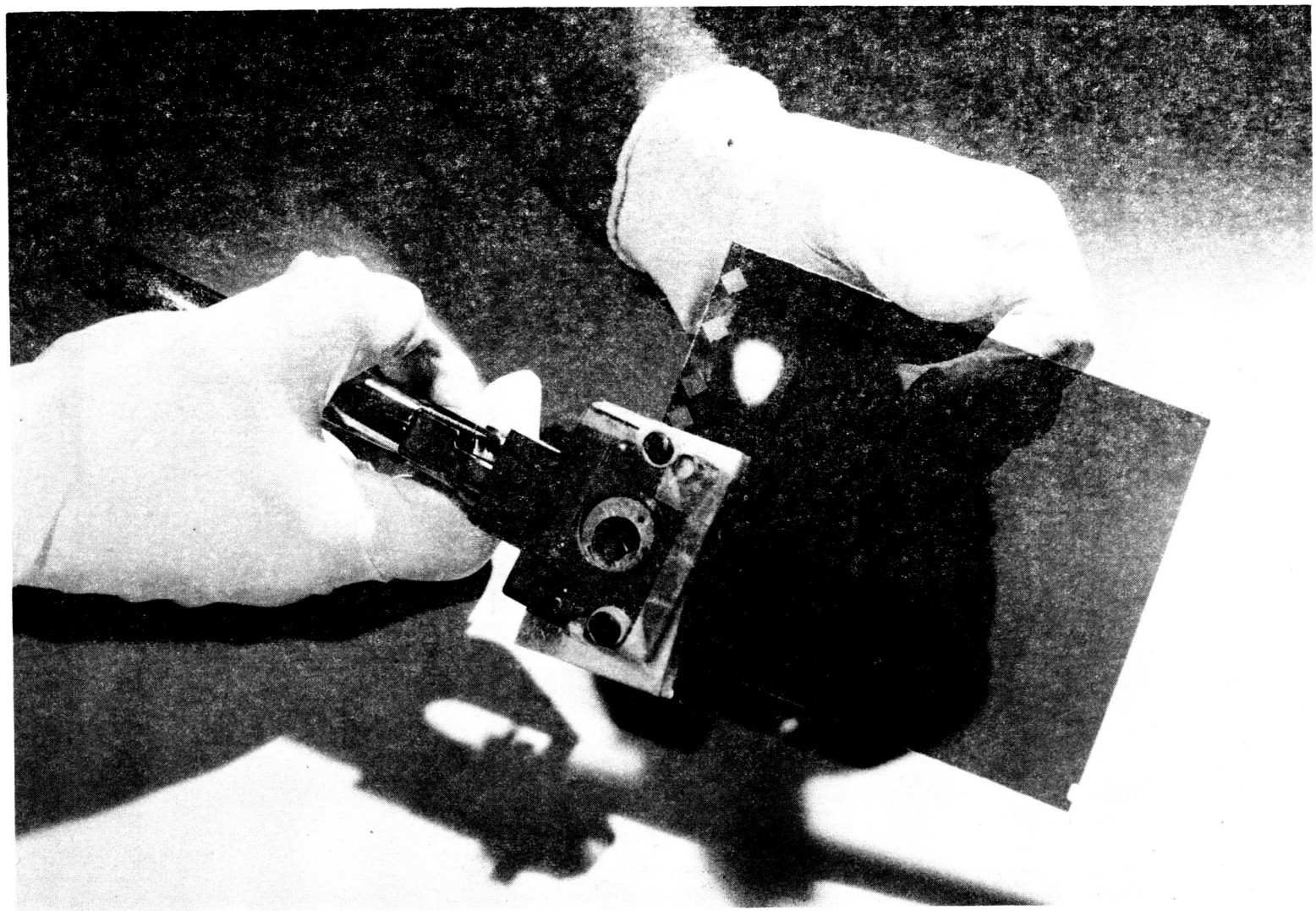

Figure 2.- High-resolution film chip punch.

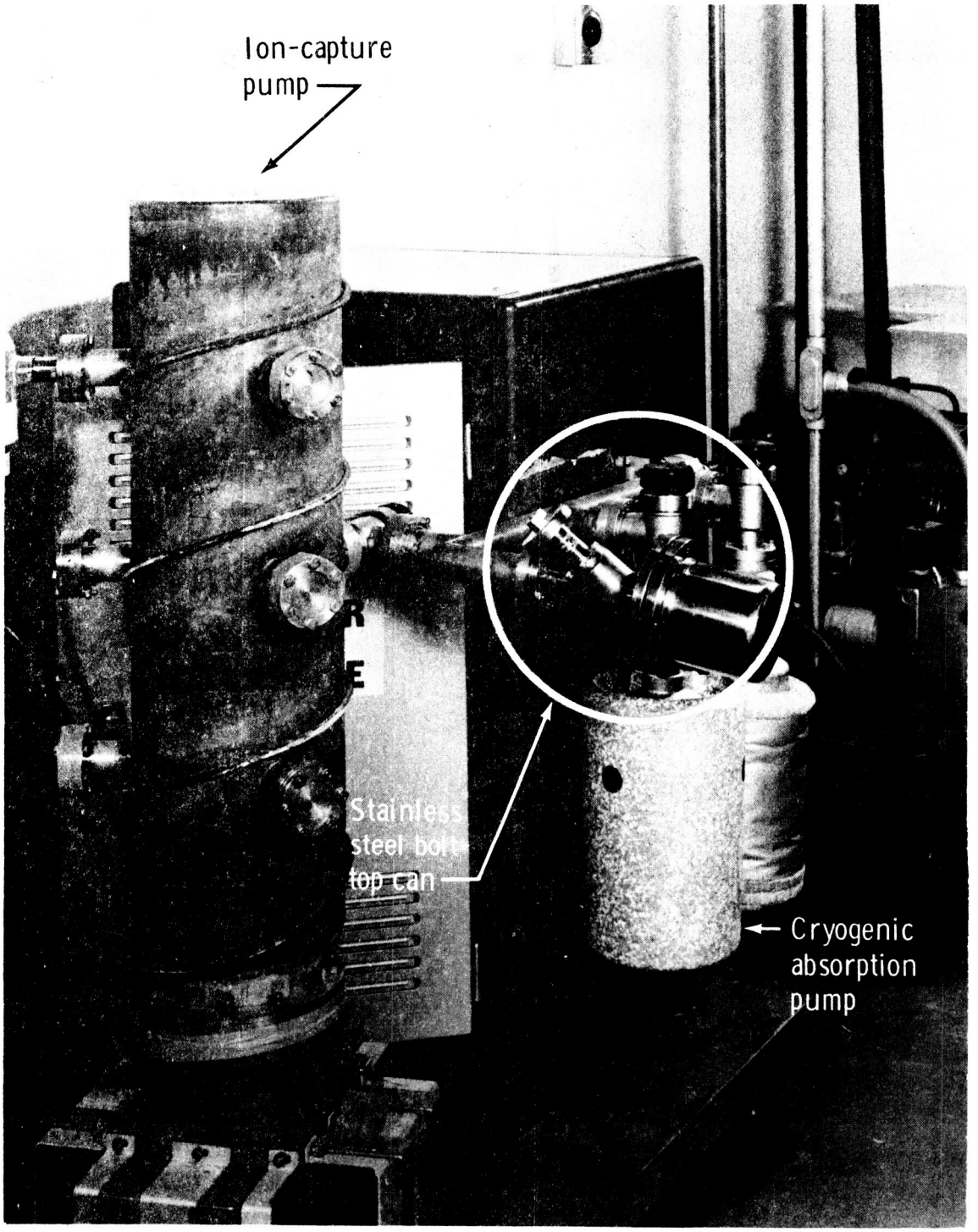

Figure 3.- Film sensitizing vacuum unit for Microbial Response to Space Environment Experiment (M191).

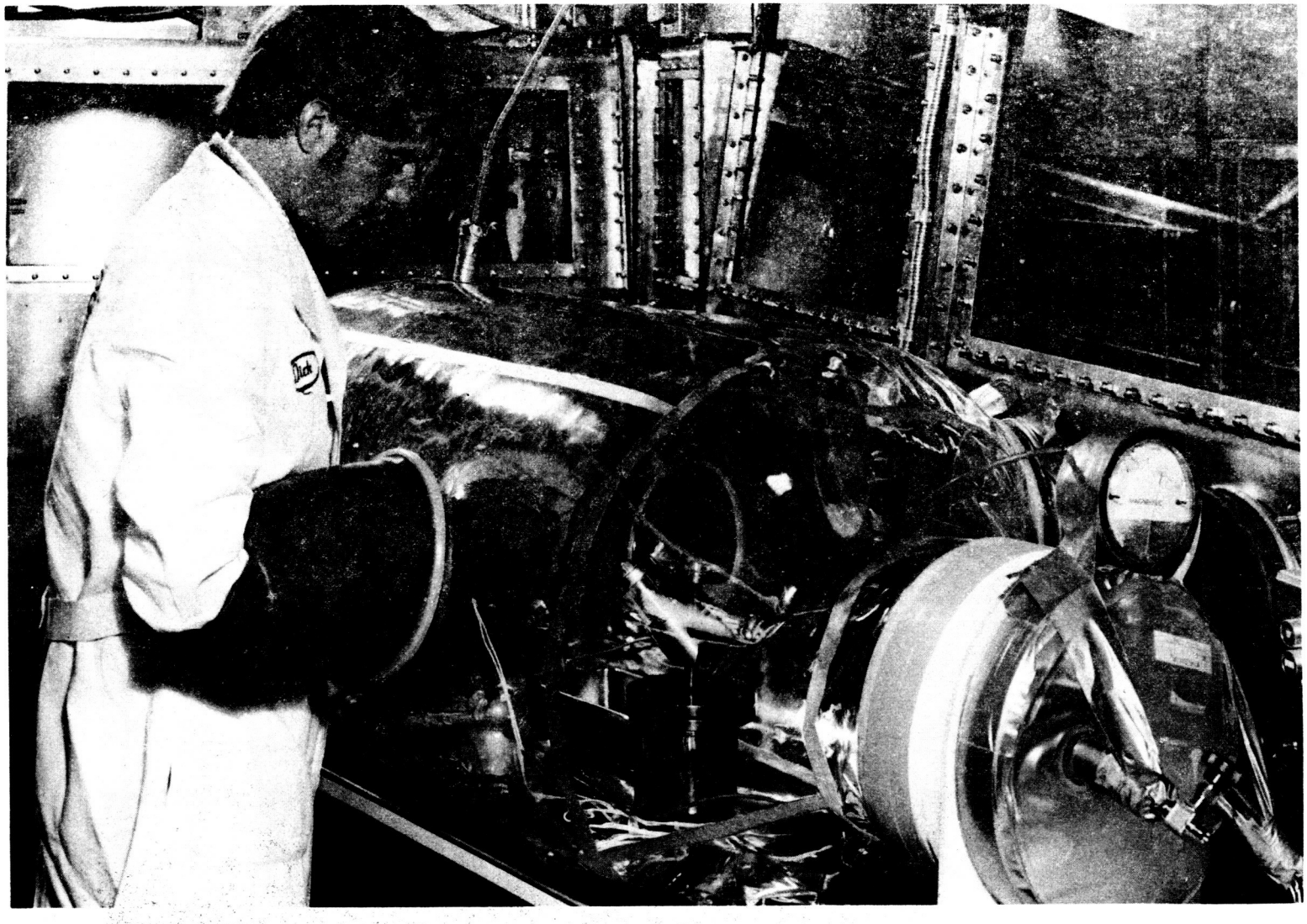

Figure 4.- Film cuvette loading chamber.

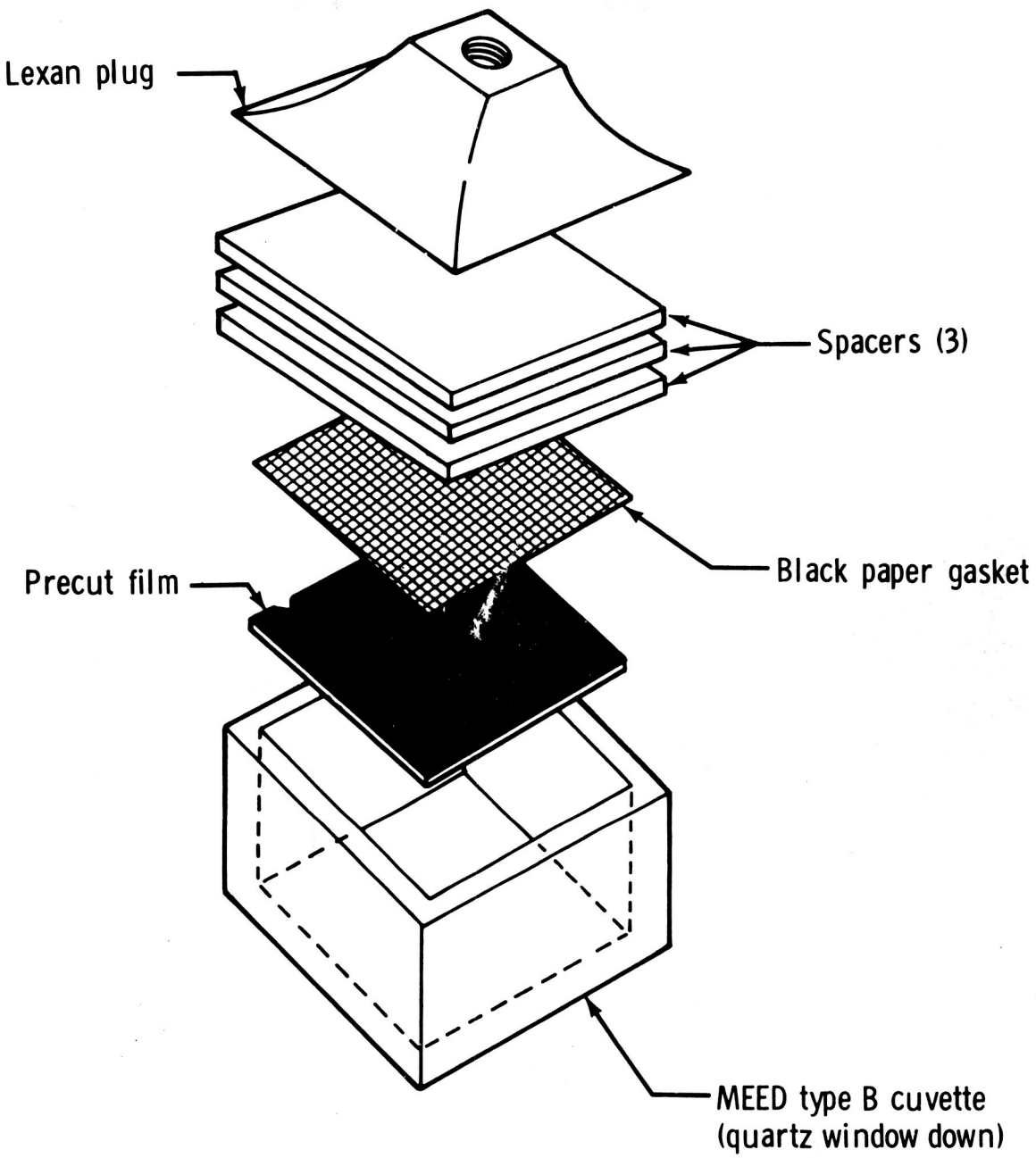

Figure 5.- Film cuvette assembly.

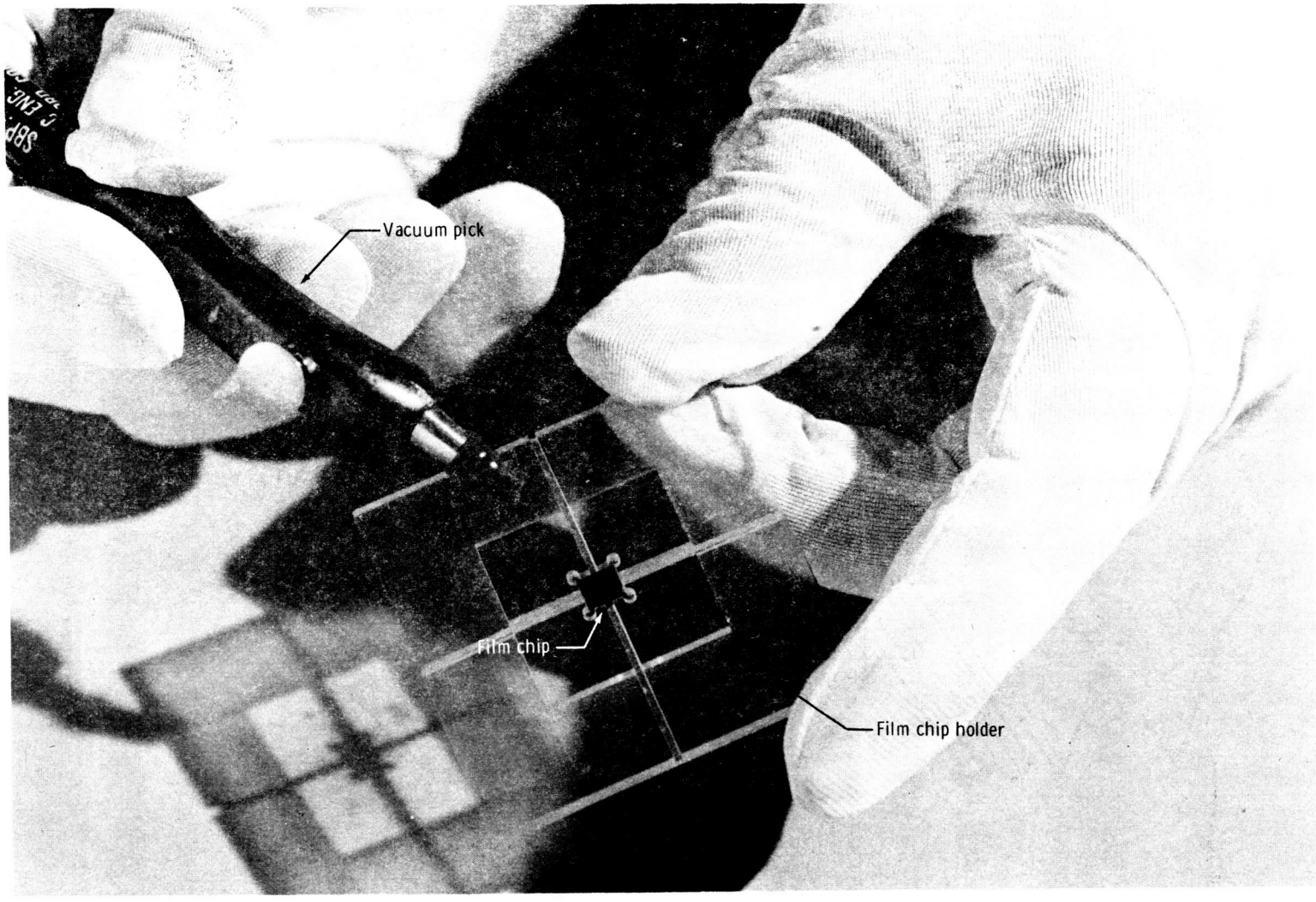

Figure 6.- Vacuum pick and film chip.

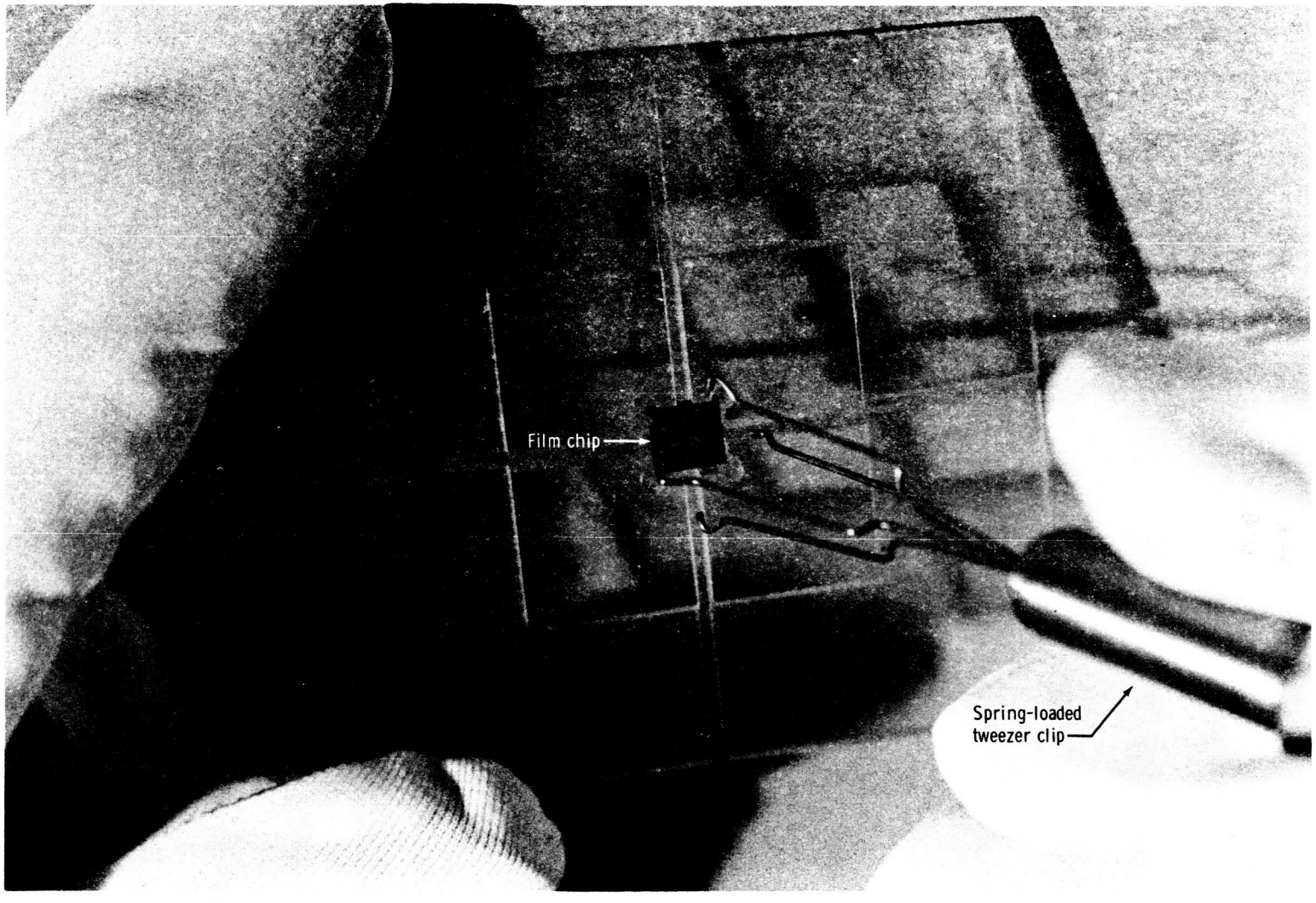

Figure 7.- Film chip transfer plate.

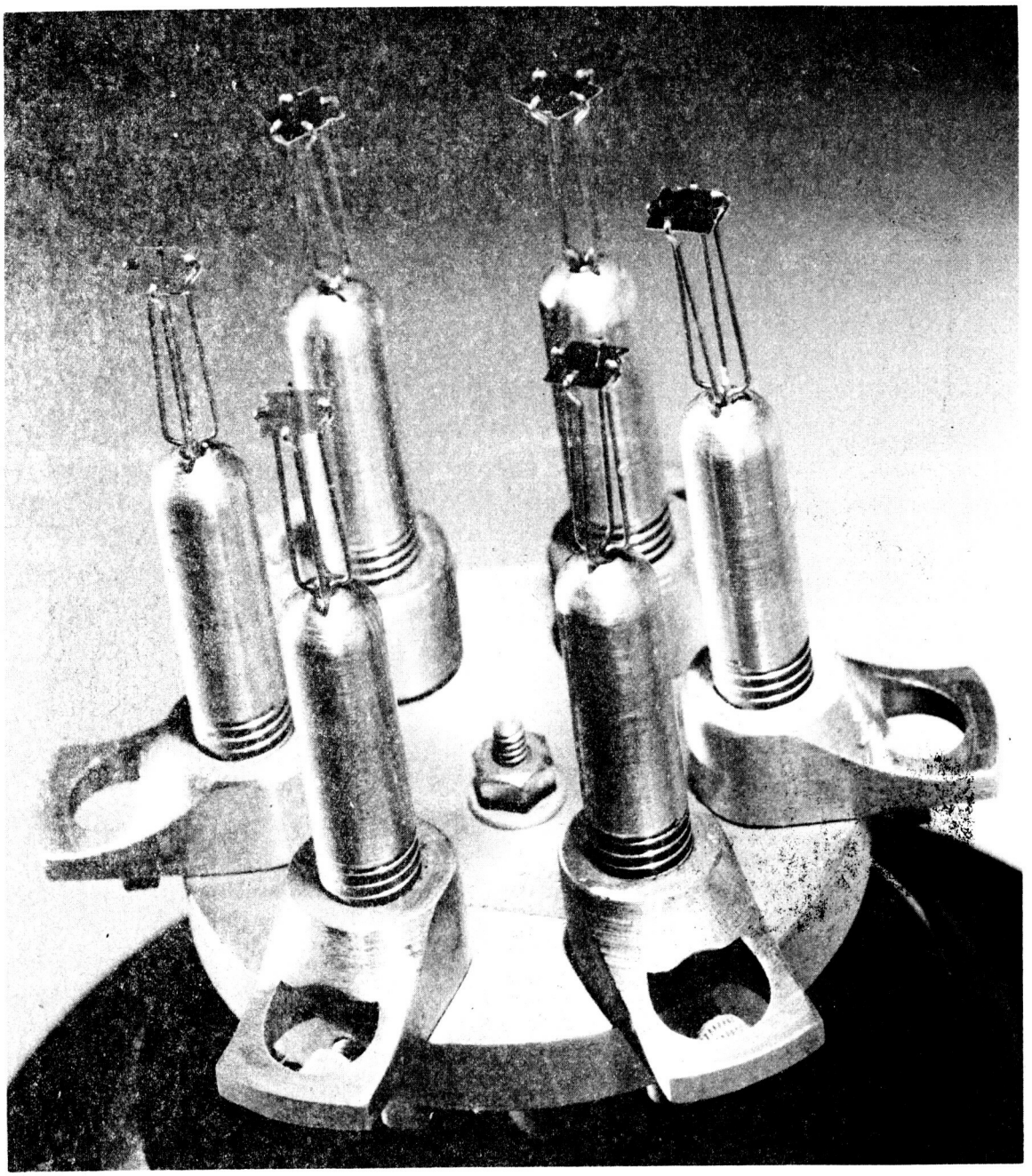

Figure 8.- Film processing carrousel.

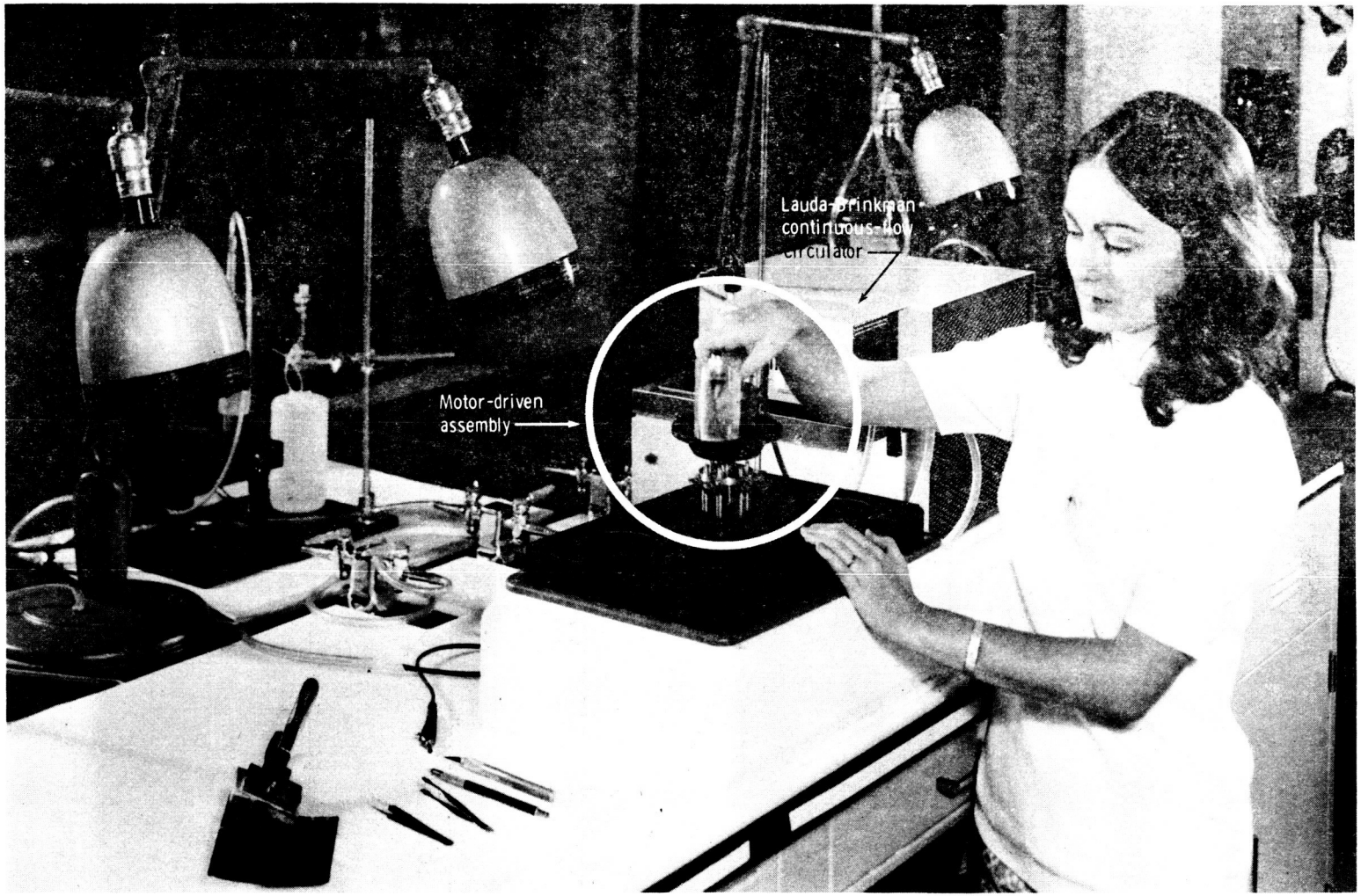

Figure 9.- Actinometry laboratory.

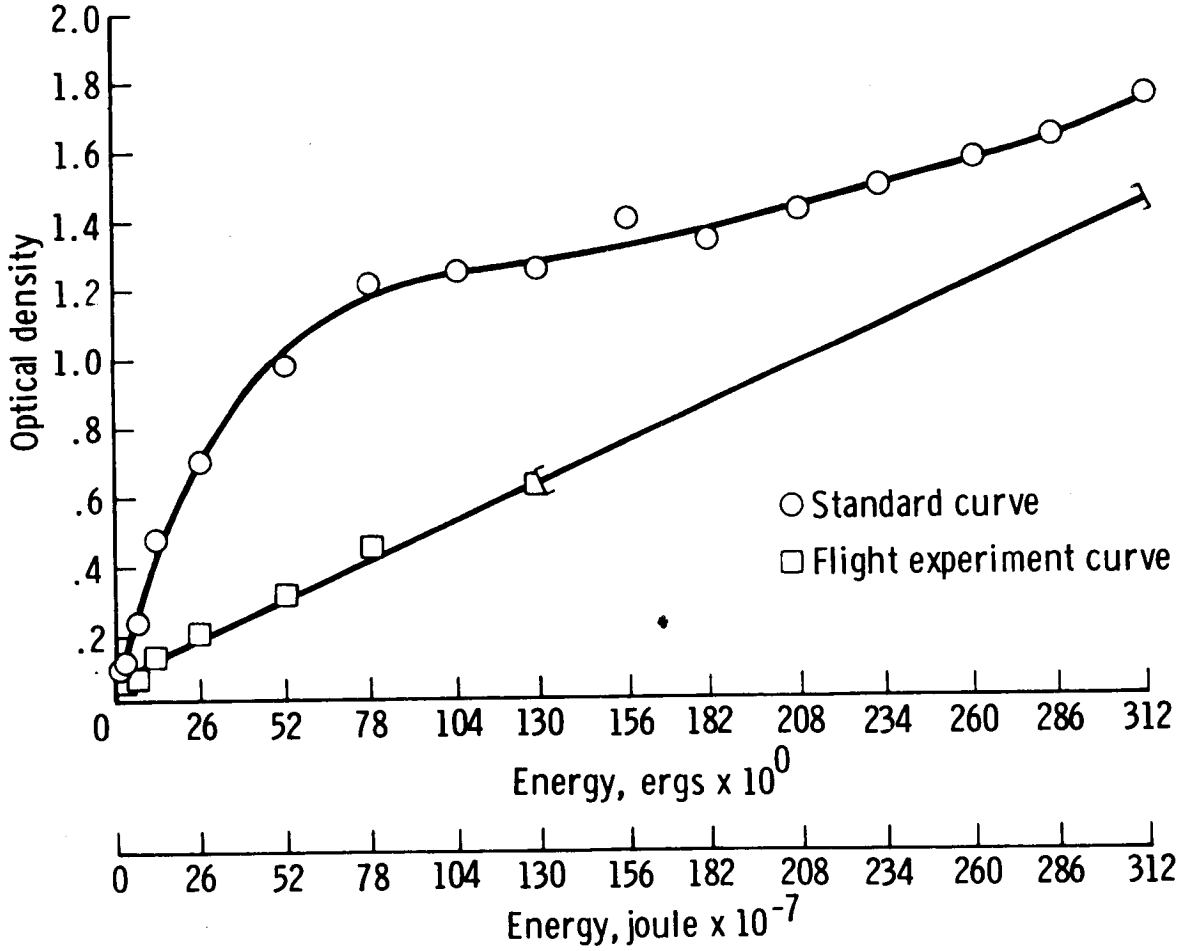

Figure 10.- Response of high-resolution film to uv irradiation at a wavelength of 254 nanometers.

THE FERRIOXALATE ACTINOMETRY SYSTEM OF THE MICROBIAL RESPONSE TO SPACE ENVIRONMENT EXPERIMENT (M191)

By Michael Parson*

ABSTRACT

The fluid actinometry portion of the Microbial Response to Space Environment Experiment (M191) was designed for measurement of the solar energy that penetrates certain optical filter systems during exposure in space. Potassium ferrioxalate was used to measure energy at peak wavelengths of 254, 280, and 300 nanometers because of its high degree of sensitivity and its linear response to the middle ultraviolet regions.

SUMMARY

The potassium ferrioxalate actinometry system was included in the Microbial Response to Space Environment Experiment (M191) to meet two objectives. The first objective was to verify the performance of the optical filter system in flight. On the basis of performance data, this system is useful both for verifying nominal filter-pack operation and for indicating aberrant conditions. The second objective was to evaluate the applicability and reliability of the optical filter system as a space flight-ultraviolet irradiation dosimeter. Comparisons of preirradiated, inflight-irradiated, and nonirradiated samples result in confirmation of the performance of this system for future space flights.

INTRODUCTION

Between 1952 and 1956, Hatchard and Parker did research on the potassium ferrioxalate system and proposed an equation for the reaction, in which light energy reduced iron (Fe) from Fe[III] to Fe[II] (ref. 1). Hatchard and Parker found that the sensitivity of the reaction was altered

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by changes in pH and temperature. By controlling these parameters, relatively accurate determinations of the quantum yield (the rate of Fe[II] production/quantum of light) could be made for particular wavelengths. The quantum yield varied at a wavelength (λ) greater than 300 nanometers, but remained constant below that wavelength (table I and ref. 2). This high degree of sensitivity and linear response to the middle ultraviolet region (ref. 3) is an extremely useful condition when measuring energy bands at wavelengths of 254, 280, and 300 nanometers.

Much of the experiment design of the fluid actinometry system was based on experimental observations at the laboratories of the hardware manufacturer and laboratories at the NASA Lyndon B. Johnson Space Center. Comparisons were made of two methods of preparation of actinometry fluid. A stoichiometric preparation was selected rather than the commonly used crystalline preparation method. The stoichiometric preparation proved to be as responsive as the crystalline preparation method and was prepared more easily. Reproducibility studies are indicative that the stoichiometric preparation and the proposed microanalysis system were reliable. The results of temperature and pH studies were similar to data from studies by Hatchard and Parker (ref. 1).

PREFLIGHT CALIBRATION METHODS AND RESULTS

The maximum and minimum detectable energy levels for 0.05 cm^3 (50 microliters) of the ferrioxalate solution were established by dose range studies. The upper limit was near 4×10^{-2} joule (4×10^5 ergs), and the lower limit was near 5×10^{-4} joule (5×10^3 ergs). The effective energy range to be used and the fact that this system could not be used with optical filters designed to transmit less than 5×10^{-4} joule (5×10^3 ergs) during the nominal 10-minute exposure were established.

The apparent decay of the Fe[II] product greatly influenced the experiment design. Although Hatchard and Parker had reported overnight stability of irradiated solutions (ref. 1), a 20-percent loss of measurable Fe[II] after 21 days was reported in studies conducted by the author. Decay tests were conducted to determine the rate of loss of Fe[II]. The increase in temperature and pH increased the rate of loss of measurable Fe[II]. Other tests were indicative that the irradiated fluid was most stable at a pH of 1.5 ± 0.5. By adjusting the pH of the solution to within this limit and by performing decay studies at several temperatures, data were collected to plot an Arrhenius curve, in which the time-related loss of Fe[II] was dependent on temperature (fig. 1). Theoretically, as long as the average temperature was known for control and

experimental solutions, rate constants could be found to express solution decay. By using these values, compensations could be made for the time-caused loss of Fe[II] at a certain temperature (ref. 4).

Decay data from a mission simulation test that used the X-25 solar source at the Jet Propulsion Laboratory proved the critical nature of the Fe[II] decay. Ferrioxalate-filled cuvettes were placed under filter packs that were designed to deliver energies of approximately 5×10^{-4} joule (5×10^{3} ergs). Most of the Fe[II] was lost during the required post-exposure delay. This loss necessitated further limitations in filter-pack selection.

In part, the high degree of sensitivity of the ferrioxalate system was caused by the degree of accuracy obtained in measuring the ferrous ion product. Light energy at the rate of 1.26 moles/quanta reduces Fe[III] to Fe[II]. Then, Fe[II] can be added to 1,10-phenanthroline at low pH to form a colored complex. The degree of color is analyzed by the use of a spectrophotometer; then, the optical density readings are translated into moles of Fe[II] by the use of a calibration curve. Moles of Fe[II] are converted to the equivalent energy by applying the quantum yield value (ref. 2).

The calibration curve was prepared before the cuvettes were loaded (fig. 2). This curve represents the response of the spectrophotometer to increasing molar quantities of Fe[II]. Increasing aliquots of a 0.400×10^{-3}-mole ferrous sulfate solution were mixed with the buffered phenanthroline reaction mixture. The resulting optical densities were read at a wavelength of 510 nanometers using microliter spectrophotometer cuvettes. A plot of the optical-density response of Fe[II] to the various molar concentrations is presented in figure 2. The calculated molar extinction coefficient (the slope of the curve) was 10 879. This value is needed to convert optical density into moles of Fe[II].

MISSION METHODS

The ferrioxalate solution was prepared stoichiometrically by diluting 2 cm^3 (2 milliliter) of a 0.2-mole ferric ammonium sulfate solution and 2 cm^3 (2 milliliters) of a 0.6-mole potassium oxalate solution with 50 cm^3 (50 milliliters) of 0.1N sulfuric acid. Irradiated decay controls were prepared on April 6, 1972, by irradiating 100 flight-approved cuvettes by the use of a germicidal lamp. Immediately, 28 cuvettes were analyzed to determine an energy baseline. Thirty-six cuvettes were placed in a bolt-top can (BTC) that was immersed in a 293° K (20° C) water bath;

five cuvettes from this group of 36 were analyzed on each of five sample periods selected at 4-day intervals. The remaining 31 cuvettes were to be analyzed with the flight-exposed cuvettes. The remaining 36 irradiated controls were divided among the ground, flight, and vibration control portions of the flight hardware. Twelve unirradiated control cuvettes were placed in each control tray. Twenty-six unirradiated cuvettes were placed in the unvented portion of the vented tray, and 13 unirradiated cuvettes were placed in the unvented tray. All manipulations of the ferrioxalate solution were performed under Kodak darkroom safe-light filter 1. The room temperature was kept at 293° ± 1° K (20° ± 1° C) at all times. On the afternoon of inflight exposure, 40 more cuvettes were irradiated in the same manner as were the cuvettes in the previous decay experiment. Ten of these cuvettes were analyzed immediately to determine an energy baseline. The remaining cuvettes were placed in the 293° K (20° C) BTC and held for analysis with the flight-exposed cuvettes.

The flight-exposed cuvettes and all related controls were analyzed between May 3 and 9. A 0.045-cm^3 (45 microliter) aliquot was extracted from each cuvette and allowed to react with a 0.4-cm^3 (400 microliter) aliquot of the buffered phenanthroline mixture. This solution was placed in a microliter cuvette for optical-density comparison with an actinometry reference. The reference was prepared by combining a 0.4-cm^3 (400 microliter) aliquot of reaction mixture with a 0.045-cm^3 (45 microliter) aliquot of actinometry solution from a nonirradiated cuvette held at 293° K (20° C). The optical-density readings were converted to moles of Fe[II] in the irradiated sample by the use of the following equation (ref. 4).

$$\text{Moles of Fe[II] in sample} = \frac{\text{O.D. } (0.445)\left(51 \times 10^{-6}\right)}{E\ (0.045)} \qquad (1)$$

where O.D. is the optical density of sample (arbitrary units), 0.445 is the total volume of ferrioxalate-reaction mixture (milliliters), 51×10^{-6} is the total volume of cuvette (liters), E is the molar extinction coefficient (10 819 O.D. units/mole/liter), and 0.045 is the total irradiated solution analyzed (milliliters). Then, the total number of moles of Fe[II] in the irradiated sample for all flight-exposed cuvettes was multiplied by the correction factor for loss of Fe[II].

$$\text{Correction factor} = e(0.0005)(r)(t) \qquad (2)$$

where e is the base of natural logarithms, 0.0005 is the rate constant at 293° K (20° C) obtained from figure 1, r is the ratio of the rate constant at 293° K (20° C) to the rate constant of the average temperature of the sample, and t is the time (hours) between irradiation and analysis. Then, the resultant value should be equal to the total number of moles of Fe[II] that was produced during irradiation. This value was converted to the number of Einsteins absorbed using the quantum yield value.

$$\text{Einsteins absorbed} = \frac{(\text{moles of Fe[II] found})}{1.26 \text{ moles/Einstein}} \tag{3}$$

The total number of ergs absorbed by the solution was obtained by applying the conversion factor for the appropriate wavelength (ref. 2).

$$\text{Ergs} = \text{Einsteins}(\text{ergs/Einstein at } \lambda) \tag{4}$$

For λ = 300 nanometers, energy absorption = 3.99×10^{12} ergs/Einstein;
for λ = 280 nanometers, energy absorption = 4.28×10^{12} ergs/Einstein; and
for λ = 254 nanometers, energy absorption = 4.71×10^{12} ergs/Einstein.

RESULTS AND DISCUSSION

Postflight analyses of the contents of returned cuvettes were performed to verify that both of the stated objectives of the experiment were satisfied. The results of statistical analyses of irradiated ground control data indicate a variation of ±7 percent in the calculated energy levels. This small variation is considered satisfactory for the purposes of this experiment. Data collected from analysis of cuvettes in the control trays of the Microbial Ecology Evaluation Device (MEED) indicate that the fluid in the irradiated controls reacted as expected. The predicted correction factor for decay brought the irradiated controls of the vibration and flight control trays within the limits of the baseline data. Use of this correction factor demonstrated that neither the space flight nor the simulated launch vibration exerted a detectable change in the preirradiated ferrioxalate.

Application of the appropriate correction factor could not compensate for all the apparent Fe[II] decay in the irradiated ground control samples. The ground control unit sustained a higher mean temperature than the other units, although the actual value is unknown because the internal

thermometers measured only the temperature extremes. The applicability of the ferrioxalate system to space-flight use was verified by the data presented. Of the two nonflight controls that were used, the one for which the temperature profile matched most closely that profile sustained ultimately by the flight hardware produced results that were not different statistically from the inflight data. In addition, the system was sensitive enough to pick up the slight temperature differential undergone by the other nonflight controls.

The cuvettes that were exposed inflight were arranged in groups of four cuvettes under a particular filter combination. In five of eight such groups, the four cuvettes yielded almost identical results. More aberrant readings were obtained from the remaining three groups. These readings are considered to be indicators of optical pressure lines formed by the 7.9×10^{-3}-centimeter-gram (100 inch-ounce) closure force that was used to assemble the flight hardware.

In general, the postflight analysis of the flight-exposed ferrioxalate cuvettes resulted in calculated energies that were higher than those anticipated (ref. 5). No absorbed energy was detected in the 11 ferrioxalate cuvettes that were placed under filter packs which were rated to transmit less than 3×10^{-2} joule (3×10^{5} ergs). This absence of absorbed energy was expected because the energy was less than that required to compensate for the expected Fe[II] loss. These cuvettes were used only to verify the lower detection limit of the system. In 14 cuvettes, absorbed energies were higher than, but relatively close to, the expected dose. In the remaining 15 cuvettes, calculated energies were from two to three times the anticipated quantities. Anomalies such as air bubbles, layering effects of the Fe[II] product, and decay of Fe[II] have been eliminated as perturbating factors. These aberrant values could be caused by an unusually high efficiency in transmission through the filter pack, by pressure lines, by variations in filter ratings, or by minor light leaks around the edge of individual filters. Clarification of the actual cause of these variations is dependent on further evaluation of the filter packs that were used.

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TABLE I.- QUANTUM YIELD VALUES AT SELECTED WAVELENGTHS OF SOLAR ENERGY[a]

Wavelength, nm	Quantum yield, moles/Einstein
505	0.85
435	1.11
405	1.16
366	1.26
.334	1.26
313	1.26
300	1.26
254	1.26

[a]Data from reference 2.

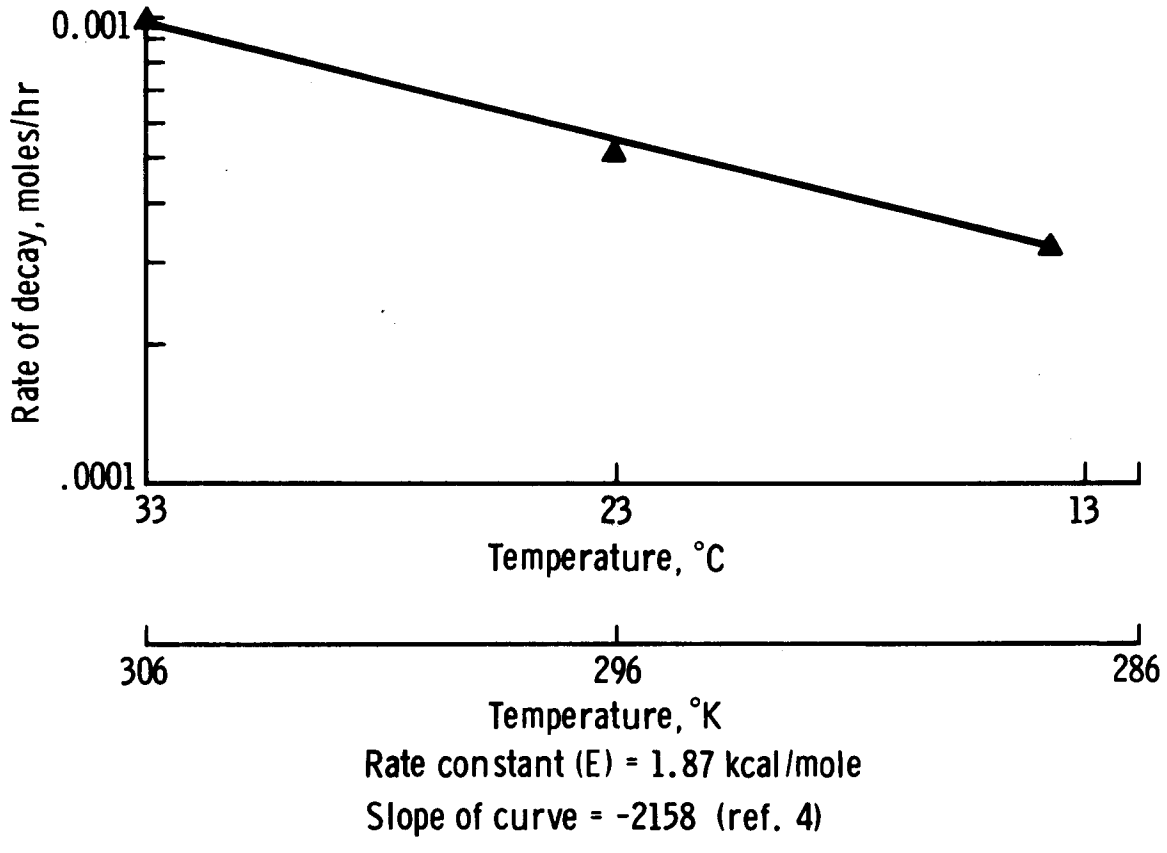

Figure 1.- Effect of temperature on the rate of Fe [II] decomposition in 0.008 M ferrioxalate solution at pH 1.6.

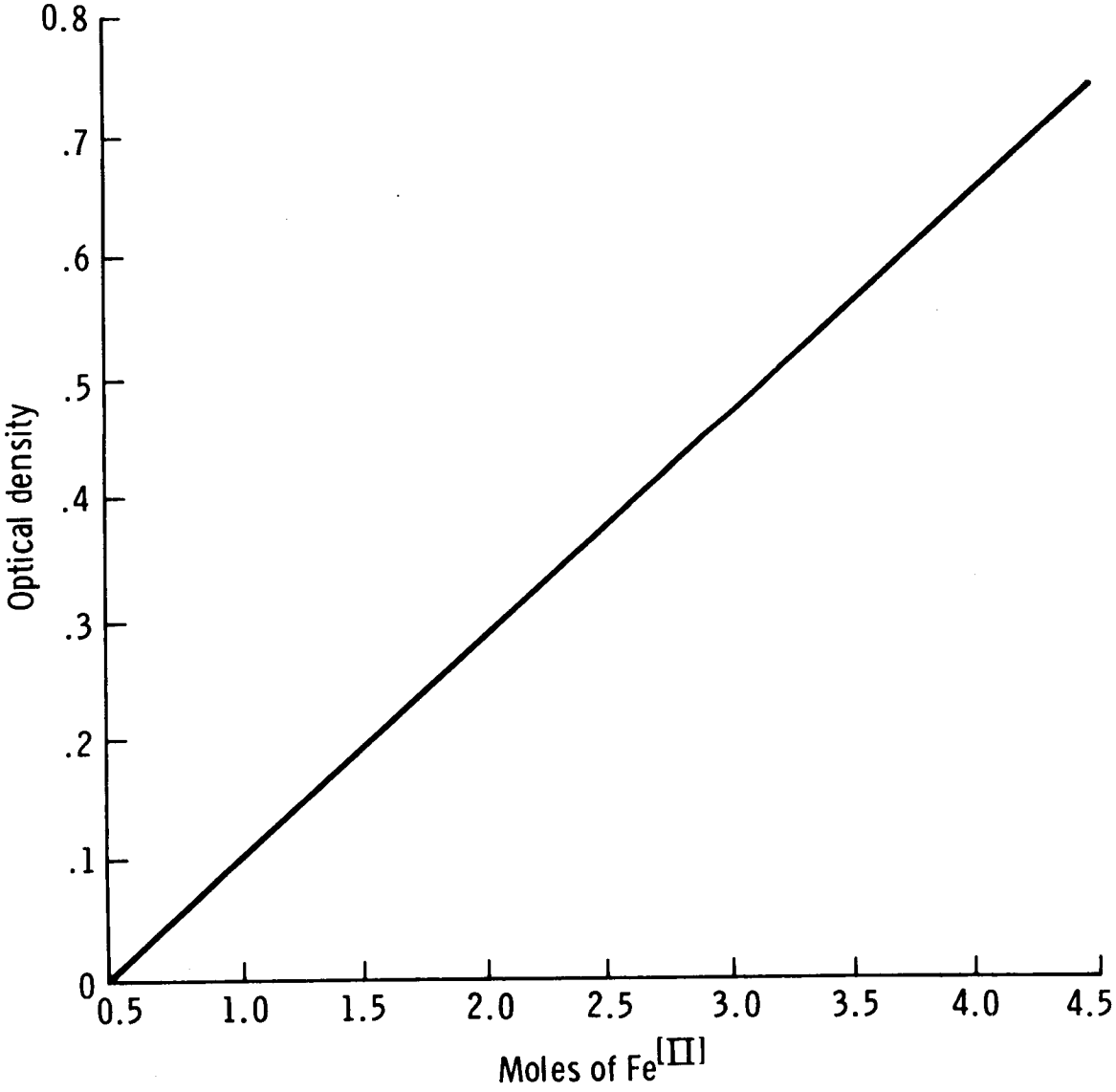

Figure 2.- Typical spectrophotometric calibration curve for ferrous sulfate.

THE HIGH-ENERGY MULTICHARGED PARTICLE EXPOSURE OF THE MICROBIAL ECOLOGY EVALUATION DEVICE ON BOARD THE APOLLO 16 SPACECRAFT

By Eugene V. Benton* and Richard P. Henke*

ABSTRACT

The high-energy multicharged cosmic-ray-particle exposure of the Microbial Ecology Evaluation Device package on board the Apollo 16 spacecraft was monitored using cellulose nitrate, Lexan polycarbonate, nuclear emulsion, and silver chloride crystal nuclear-track detectors. In this report, the results of the analysis of these detectors include the measured particle fluences, the linear energy transfer spectra, and the integral atomic number spectrum of stopping particle density. The linear energy transfer spectrum is used to compute the fractional cell loss in human kidney (T1) cells caused by heavy particles that have a linear energy transfer that is greater in value than 1.6×10^{-14} joule cm^2/mg (0.1 MeV cm^2/mg). Because the Microbial Ecology Evaluation Device was better shielded, the high-energy multicharged particle exposure was less than that measured on the crew passive dosimeters.

SUMMARY

The exposure of the Microbial Ecology Evaluation Device package on board the Apollo 16 spacecraft to high-energy multicharged particles was measured by use of a variety of nuclear-track detectors. Results are presented in the form of measured particle fluences, the linear energy transfer spectra, and the integral atomic number spectrum of stopping particle density. Excellent agreement was found between the linear energy transfer spectra measured with Lexan and cellulose nitrate detectors. Because of the more effective shielding on the Microbial Ecology Evaluation Device package, the high-energy multicharged particle exposure was approximately four times smaller than the exposure on the crew passive

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dosimeters. The data presented in this report can be used to evaluate the various possible biological endpoints that resulted from the high-energy multicharged particle exposure after the relevant cross sections for the endpoints became known.

INTRODUCTION

The objective of this study was to evaluate the exposure of the Microbial Ecology Evaluation Device (MEED) to the highly ionizing, high-energy multicharged component (HZE) of the cosmic-ray-particle spectrum. In view of the complexity of the shielding, which has a pronounced effect on the heavy-radiation component (refs. 1 and 2), the heavy-particle flux was monitored using various solid-state nuclear-track detectors that were included as an integral part of the MEED package. The detectors included Lexan polycarbonate, cellulose nitrate (CN), Ilford G5 emulsions, and silver chloride (AgCl) crystals. The tissue equivalent detectors, Lexan and CN, were used to measure the particle linear energy transfer (LET_ω) spectrum (ref. 3), which probably provides the simplest complete description of the biological effectiveness of the heavy-radiation component. The emulsions and the AgCl crystals served as backup detector systems.

This report primarily deals with the LET_{350} spectrum as measured in both the Lexan polycarbonate and the CN plastic detectors. From the combined results of the measurements in these two plastics, the fractional cell loss, computed by use of the inactivation cross sections of Todd (ref. 4), is given as an illustration of how the LET spectrum can be used to obtain results with direct biological endpoints. The integral atomic number (Z) spectrum of stopping particle density is presented also. This more complete description of the radiation exposure can be used for Z-dependent processes. For example, to convert an LET_ω spectrum to one with a different value of ω, the process is slightly Z-spectrum dependent.

MATERIALS AND METHODS

The MEED dosimetry package, which has an area of approximately 7 cm^2, consisted of two nuclear emulsions (each 200 micrometers thick) of Ilford G5 on a Melinex base, 10 CN layers (approximately 200 micrometers thick), six Lexan layers (190 micrometers thick), and a small AgCl crystal. All detectors were encased in a small plastic box that was contained in the flight control portion of the MEED (ref. 5).

The CN layers were processed by etching them for 20 hours in 6.25 N sodium hydroxide (NaOH) at 313° K (40° C). Four of the Lexan layers were processed by sensitizing them with a 40-hour ultraviolet (uv) irradiation exposure 5.5 centimeters from the arc of a 400-watt mercury arc lamp (ref. 6) and by etching them for 8 hours at 343.4° K (70.4° C) in a 6.25 N NaOH solution with 0.5 percent Benax surfactant added.

Scanning for the etched tracks was performed under an optical microscope at 210× magnification. Measurements were made at a magnification of 530×, by use of an oil immersion objective lens with the sample immersed in water and covered by a glass cover slip.

The CN plastic response was calibrated by using stopping oxygen-16 (^{16}O) particles at various residual ranges (ref. 7). These particles were accelerated at the University of California at Berkeley Bevatron. The Lexan calibration was obtained by using ^{16}O and neon-20 (^{20}Ne) ions accelerated by the Berkeley heavy ion linear accelerator (HILAC). The CN calibrations obtained can be expressed by

$$V_T = 1.70 \times 10^4 \left(LET_{350}^{4.067} \ \mu m/hr \right) \tag{1}$$

The Lexan calibrations obtained can be expressed by

$$V_T = 182 \left(LET_{350}^{2.119} \ \mu m/hr \right) \tag{2}$$

In CN, V_T is the measured track etch rate; in Lexan, V_T is the track etch rate corrected for uv attenuation (ref. 3). The values of LET_{350} are expressed in MeV/μm in the materials in question.

To account for scanning and detector efficiency, the vertical track etch rate component was measure, and the integral distribution of this quantity was plotted on a log-log plot. The point at which this distribution deviates from a straight line with the slope theoretically derived from the calibration indicates the point at which tracks become too small to be resolved with near 100-percent efficiency by the scanner. This cut-off value of the vertical etch rate component and the number of tracks having values of the vertical etch rate greater than this cut-off value are used to obtain an objective measure of the particle fluences, the LET, and the Z spectra (ref. 3).

RESULTS

The scanned CN layer yielded 109 tracks caused by the heavy, high-energy cosmic ray particles in its 6.82-cm^2 area, yielding a measured fluence of 16.0 ± 1.5 tracks/cm^2. Of these tracks, 19 registered in the adjacent Lexan layer, yielding a measured fluence of 2.8 ± 0.6 tracks/cm^2.

Track length measurements in both detectors were reduced to integral LET_{350} spectra. The spectra as measured in CN and Lexan are shown in figures 1 and 2, respectively. A conversion was made to values in water (H_2O) by scaling the LET values to those of the same ions at the same velocity in water. This procedure is justified because of the very-near tissue equivalence of the CN and Lexan detectors. The straight-line representations of the LET spectra shown in figures 1 and 2 are the theoretical results obtained by using the cut-off track fluences described previously and by assuming that the distribution in space angles is isotropic and that the gradient of the stopping particle density is negligible in the vicinity of the detectors. The measured spectra given are obtained by converting the measured track etch rates into LET values by using the calibration data and by dividing by the solid-angle projected-area factor consistent with the previously described cut-off. The explicit mathematical representation of the theoretical LET spectrum is

$$\frac{d^2N}{dA_p\,d\Omega}\left(LET_{350,H_2O} > L\right) = C_L\,L^{-2.262} \qquad (3)$$

where dA_p is the element of projected area in cm^2, $d\Omega$ is the element of solid angle in steradians, and L is in MeV cm^2/mg. For CN and Lexan, respectively, the values of C_L are 1.56 ± 0.16 and 1.42 ± 0.35, which are indicative of good agreement between the measurements in the two plastics. The weighted average value of C_L is 1.53 ± 0.15. The uncertainties indicated in figures 1 and 2 are caused by counting statistics alone.

Equation (3) can be used to obtain objective values of the particle fluences. To obtain the fluence of particles with $LET_{350,H_2O} > L$ falling on a sphere of a 1-cm^2 projected area, equation (3) must be multiplied by 4π steradians; to obtain the fluence passing through a 1-cm^2 planar area,

equation (3) must be multiplied by 2π (the average projection factor is 0.5). Thus, the spherical fluence of particles with $LET_{350,H_2O} >$ 1 MeV cm^2/mg is 19.3 ± 1.8 particles/cm^2. The planar fluence with $LET_{350,Lexan} > 0.15$ MeV/μm is 4.9 ± 0.5 particles/cm^2. The arbitrary cut-off value of $LET_{350,Lexan} = 0.15$ MeV/μm is in accord with previous usage and is used here to allow objective fluence comparisons.

When the cross-sectional area for some biological process is known as a function of LET, this cross section times the differential form of equation (3) can be integrated to obtain the occurrence frequency of this particular endpoint. An example is the computation of the fractional cell loss (FCL) for human kidney (T1) cells in vitro from the cross section per cell for irreversible cell inactivation given by Todd (ref. 4). To match the independent variable of Todd's measurements, equation (3) is differentiated and converted to an LET_∞ spectrum (ref. 3) by using the stopping particle density Z spectrum (fig. 3). The results of the integration with the inactivation cross sections are given in figure 4.

The density of stopping particles with $Z > Z_0$ was established using the Z distribution of particle fluences given in reference 3. All particle charge measurements have been rounded to the most likely even Z value, keeping in mind the measurement inaccuracy and the much greater frequency of the even compared with the odd Z nuclear cosmic-ray-particle species. The results are presented in figure 4.

DISCUSSION

It is not fortuitous that the agreement between the CN and Lexan LET spectra is much better than expected from the counting statistics. This agreement is because the same group of tracks passes through both plastics and counting statistics are not involved in the comparisons, except for tracks with very small dip angles. The probable sources of error are the small number of tracks observed in Lexan, the possible inaccuracy in the calibrations, and the inaccuracy in the assumptions about the space angle and particle-stopping-point distribution. In fact, the first source of error could account for the entire discrepancy in the results if only 1.6 more tracks had been recorded in the Lexan track count. The degree of the agreement vindicates the previously stated assumption (ref. 3) that the errors in the LET spectrum caused by counting statistics are greater than errors arising from the calibration and the assumptions about the space angle and stopping-point distributions.

The scaling of the Z spectrum is subject to greater uncertainties because the shielding in the MEED location is greater than that of the cabin itself. The additional shielding affords a preferential reduction in the heavier-particle component of the particle spectrum, largely by means of nuclear fragmentation. Scaling is justified, however, in that its only use has been to convert the measured LET_{350} spectrum into an LET_{∞} spectrum. This conversion is only slightly dependent upon the Z spectrum.

The approach used to reduce the MEED fluence measurements to absolute values represents an improvement over the previous approach of extrapolating the integral distribution in track-opening size to zero track size (ref. 3). The current approach is to use a cut-off procedure at a nonzero, known vertical etch-rate value, then to reduce to absolute fluences accordingly. This approach could not be used safely with the track-opening-size distribution because deviations from the theoretical track-geometry models (ref. 3) produce errors in the predicted track-opening-size distributions for nonzero opening.

The Apollo 16 personal radiation dosimeter average fluence of particles with $LET_{350,Lexan} > 0.15$ MeV/μm of 19.4 ± 0.8 tracks/cm^2 (ref. 9) is 4.0 ± 0.4 times larger than the corresponding MEED fluence of 4.9 ± 0.5 tracks/cm^2. Therefore, there is significantly larger attenuation of the heavy particle LET spectrum in the heavily shielded MEED package.

The quantity LET_{ω} is the energy transfer per path length to electrons receiving less than ω electron volts. For plastics, $\omega = 5.8 \times 10^{-17}$ joule (350 eV) represents the observed response well. The value of ω applicable to tissue has been assumed to be ∞ but quite possibly is different (but not measurable easily) in specific cases. The value of ω is a function of the size of the region most vulnerable to radiation damage. In the introduction, it was stated that LET_{ω} probably is the simplest complete description of the biological effectiveness of the heavy radiation component. Theoretically, it is only complete if the value of ω is the proper one for the biological process involved. However, biological measurements are rarely sufficient to reveal this distinction.

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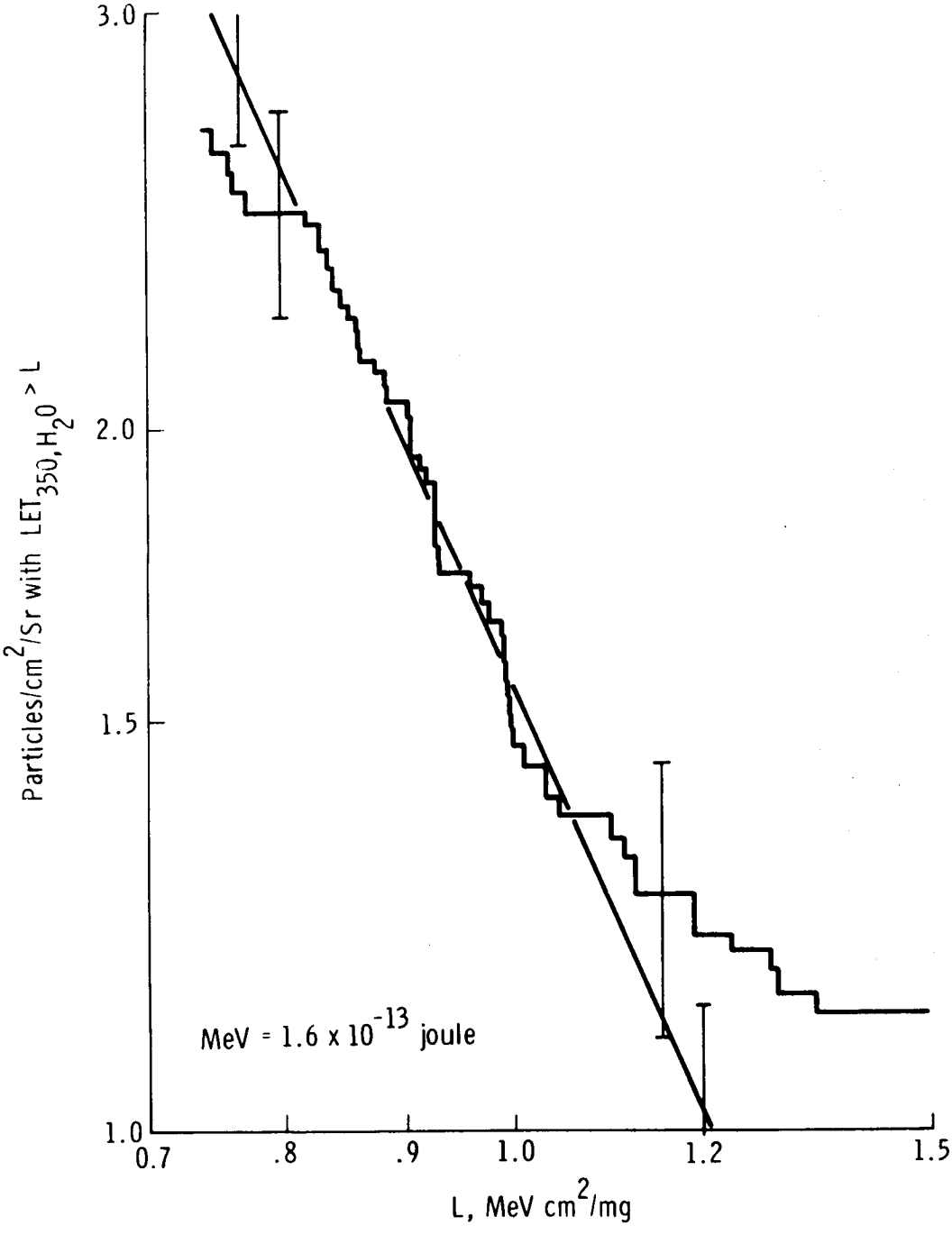

Figure 1.- The integral LET_{350} spectrum measured in CN and reduced to LET_{350} values in water. The measured spectrum and the theoretical spectrum (straight line) based on the cut-off fluence are given. The errors indicated are caused by counting statistics.

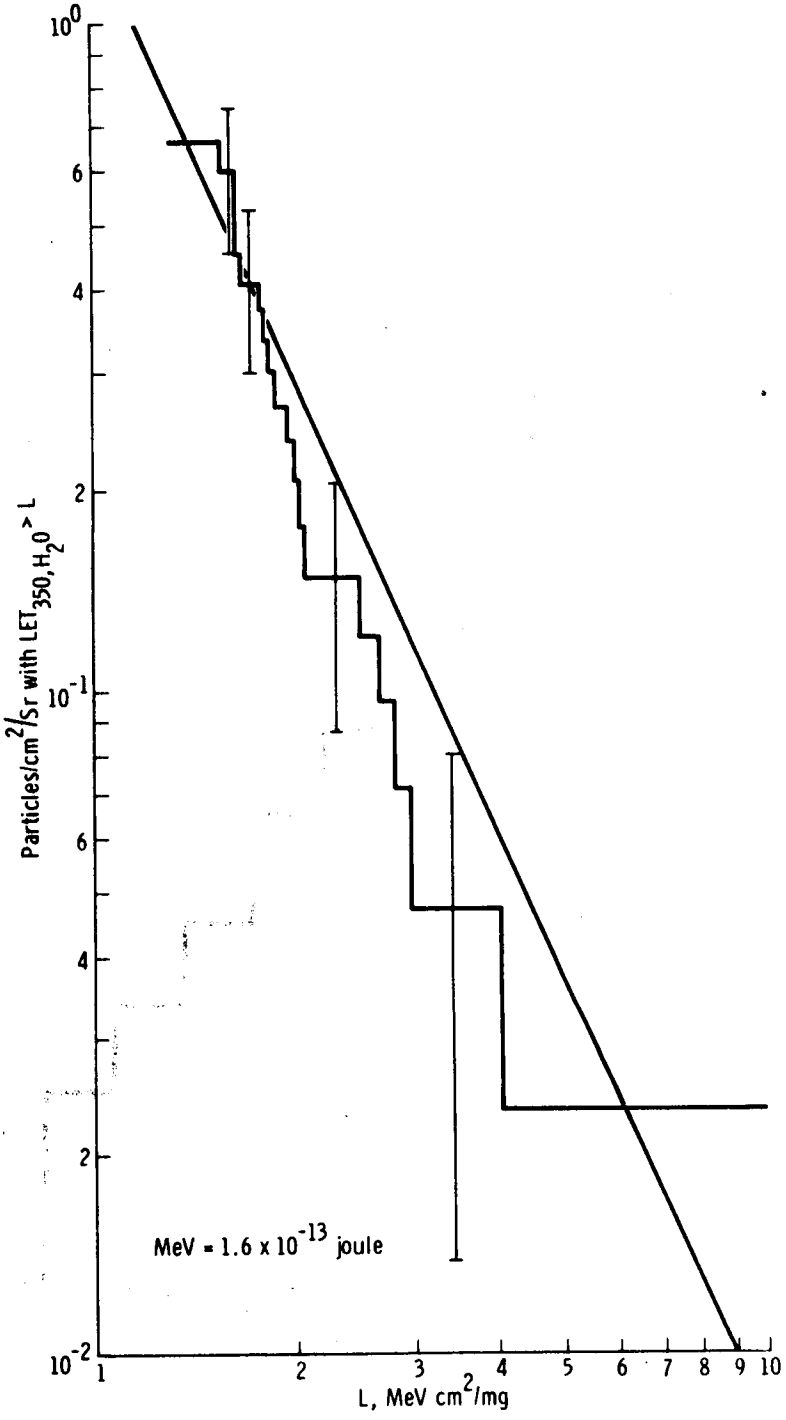

Figure 2.- The integral LET_{350} spectrum measured in Lexan and reduced to LET_{350} values in water.

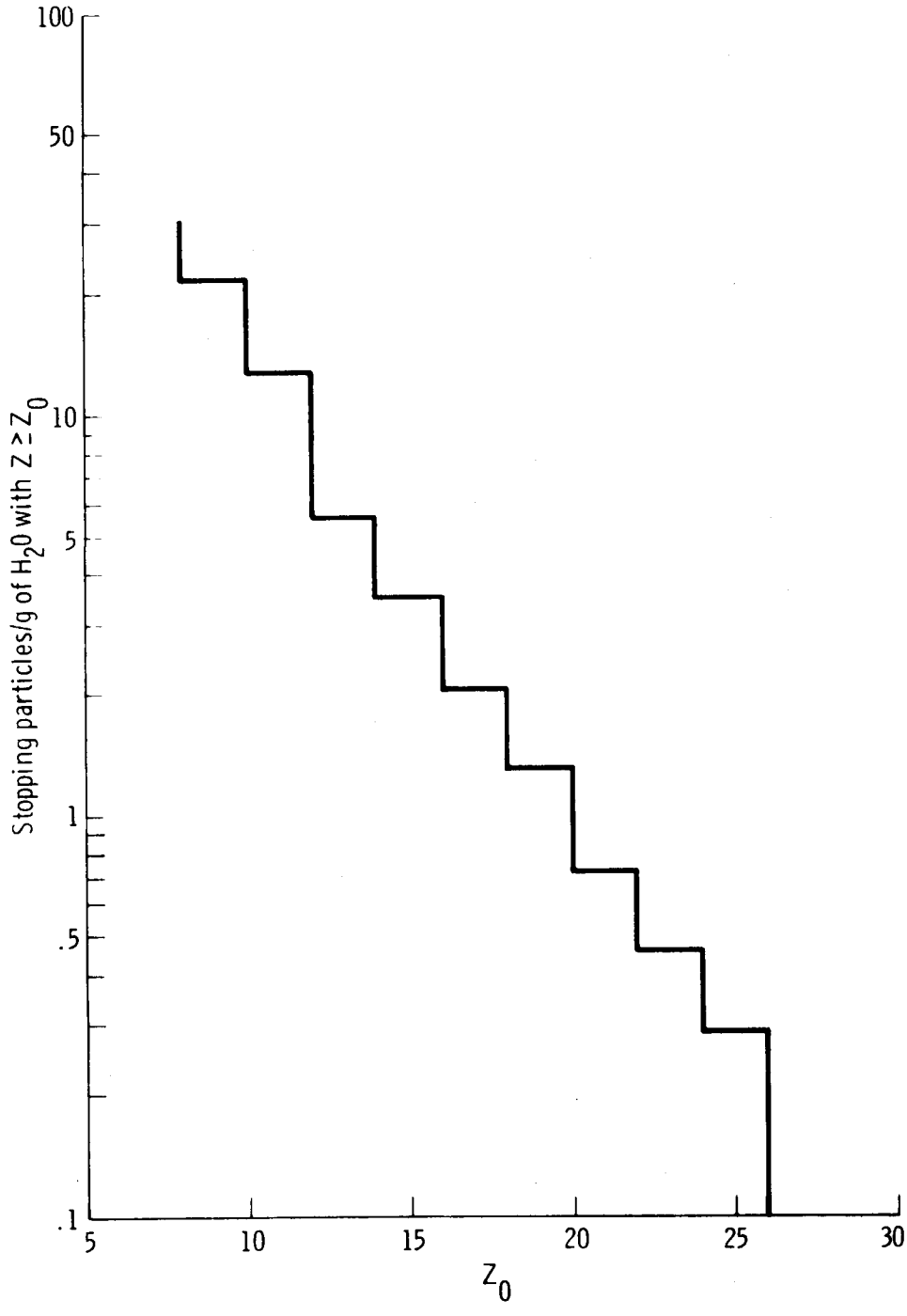

Figure 3.- The integral Z spectrum of stopping particle densities.

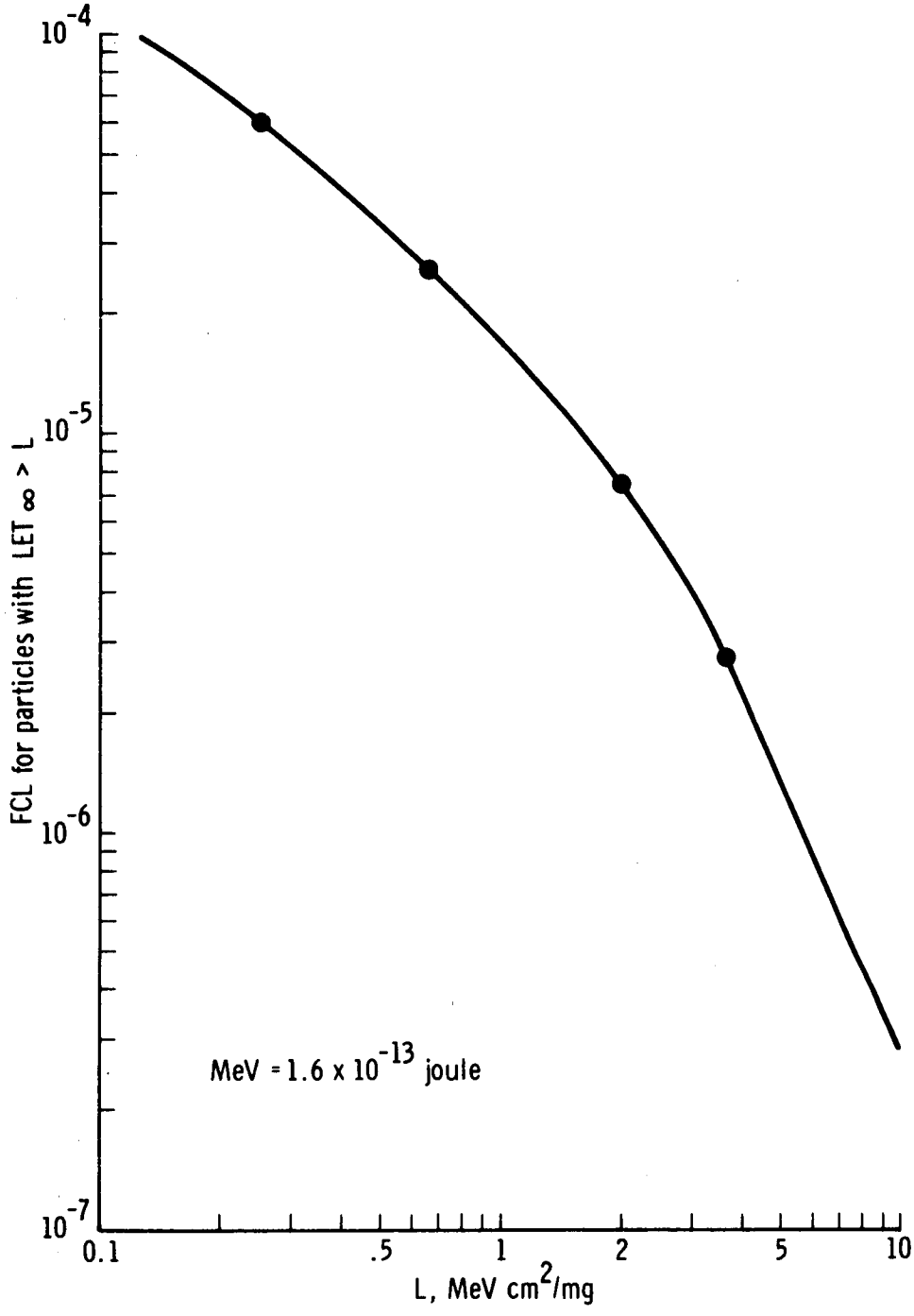

Figure 4.- The fractional cell loss (FCL) in human kidney (T1) cells caused by particles with $LET_\infty > L$. The region of the plot with $L < 0.75$ MeV cm^2/mg represents an extrapolation of the measured LET spectrum.

THERMOLUMINESCENT DOSIMETRY FOR THE APOLLO 16 MICROBIAL RESPONSE TO SPACE ENVIRONMENT EXPERIMENT (M191)

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ABSTRACT

Lithium fluoride thermoluminescent chips were used to provide an integrated dose from the broad spectrum of ionizing radiation to the Microbial Response to Space Environment Experiment (M191). The chips were positioned in the flight hardware to provide data on ionizing radiation within specific volume segments. A uniform radiation dose of $4.8 \times 10^{-3} \pm 2 \times 10^{-4}$ joule/kg (0.48 ± 0.02 rad) resulted.

SUMMARY

Postflight radiation analysis of thermoluminescent dosimeters from the ultraviolet-irradiated and flight control trays of the Microbial Ecology Evaluation Device was indicative that the ionizing radiation level was uniform throughout the flight hardware at $48 \times 10^{-4} \pm 2 \times 10^{-4}$ joule/kg (0.48 ± 0.02 rad). This dose represents an absorption of $48 \times 10^{-7} \pm 2 \times 10^{-7}$ joule (48 ± 2 ergs) of ionizing energy per gram of biological material within flight control and ultraviolet-irradiated cuvettes. An ionizing radiation dose of less than 5×10^{-5} joule/kg (5×10^{-3} rad) was recorded on ground control dosimeters. Ionizing radiation may be considered constant in terms of the effect upon flight controls compared with ultraviolet-irradiated flight materials but should be considered variable in terms of effects relative to flight control compared with ground control studies.

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INTRODUCTION

Biological materials contained within the Microbial Response to Space Environment Experiment (M191) on the Apollo 16 mission were exposed to solar ultraviolet (uv) irradiations at wavelengths of 254, 280, and 300 nanometers, and to the unfiltered solar spectrum. Also, throughout the mission, these materials were being exposed to ionizing radiations that were penetrating the spacecraft.

Lithium fluoride (LiF) thermoluminescent chips were chosen to provide quantitative measurement of the integrated dose from the broad spectrum of ionizing radiations that were present in the space environment. Included in the flight hardware were dosimeters to determine ultraviolet levels and materials to determine ionizing radiation quality. This report only deals with the broad spectrum ionizing radiation analysis provided by LiF thermoluminescent dosimetry for the Microbial Ecology Evaluation Device (MEED).

DOSIMETER SELECTION AND PREPARATION

Extruded LiF thermoluminescent dosimeter (TLD) chips were sized (5 by 5 by 1 millimeters per chip) to allow the mounting of four TLD chips per MEED cuvette. The chips were tested by the use of standard methods (refs. 1 to 4) for uniformity of response and were segregated according to relative calibration performance. Response of the chips with the best relative performance (calibration factor = 1.00 ± 0.025) was confirmed by colbalt-60 irradiation at the NASA Lyndon B. Johnson Space Center Health Physics Calibration Facility. Two hundred chips were selected from this confirmed-response group for use in the experiment. The final steps in TLD chip preparation included annealing (1 hour at 673° K (400° C) followed by 2 hours at 373° K (100° C)) and loading into cuvettes. Fifty cuvettes were loaded as follows: 19 cuvettes for mounting in the flight hardware; 21 cuvettes for vibrational testing and ground control; and 10 cuvettes for irradiation calibration. The TLD-loaded cuvettes were arranged within the hardware as shown in figure 1.

RESULTS

Radiation calibration was performed against cobalt-60 during the Apollo 16 mission. Analyses of flight chips, control chips, and calibration chips were performed 20 days after recovery. Cobalt-60 irradiation of the calibration group yielded the response shown in figure 2.

Response of controls (less than 5×10^{-5} joule/kg (5×10^{-3} rad)) was subtracted from the response of the flight group to give the results shown in table I. The error factors indicated by plus or minus values in table I represent one standard deviation (1σ) of the sample. Each cuvette represents a sample of four chips.

DISCUSSION

Cuvettes containing TLD chips were positioned to provide data on ionizing radiation dose within specific volume segments of the MEED. The chips were distributed throughout the three tray tiers as shown in figure 1. This distribution allowed dose determination for each tier, for each of the six sides of the flight hardware, and for the central volume of the closed assembly.

The ionizing radiation dose results were analyzed statistically. The flight hardware received extremely uniform irradiation from the ionizing radiation components of the space environment. Not one of the individual volume segments showed a statistically significant difference from the mean of all chips ($4.8 \times 10^{-3} \pm 2 \times 10^{-4}$ joule/kg (0.48 ± 0.02 rad)). The individual cuvette results and mean values for each tray tier (table I) are illustrative of this uniformity. The extreme limits of dose measured by TLD throughout the flight hardware are 44×10^{-4} joule/kg to 51×10^{-4} joule/kg (0.44 to 0.51 rad). Crewmen received ionizing radiation doses that ranged from 48×10^{-4} to 54×10^{-4} joule/kg (0.48 to 0.54 rad) according to the crew passive TLD measurements. Mean values were $51 \times 10^{-4} \pm 2 \times 10^{-4}$ joule/kg (0.51 ± 0.02 rad). Crew passive dosimeter and MEED cuvette TLD materials were calibrated concurrently but were analyzed independently to allow accurate and unbiased comparison of results.

The dose of $48 \times 10^{-4} \pm 2 \times 10^{-4}$ joule/kg (0.48 ± 0.02 rad) represents an absorption of $48 \times 10^{-7} \pm 2 \times 10^{-7}$ joule (48 ± 2 ergs) of ionizing energy per gram of biological material within the flight hardware. This value is applicable to all samples within the flight hardware, including flight controls and uv-irradiated samples.

Spacecraft active radiation instrumentation indicated that approximately 25×10^{-4} joule/kg (0.25 rad) of the total Apollo 16 dose originated from the protons and electrons of the Van Allen belt during the

ascent of the spacecraft through the belt at the time of translunar injection. The majority of the dose was from protons. The Van Allen belt dose was received over the relatively short period (approximately 30 minutes) at dose rates ranging to a maximum of 2.71×10^{-2} joule/kg/hr (2.71 rad/hr). The remaining dose was obtained at a rate of approximately 1×10^{-5} joule/kg/hr (1×10^{-3} rad/hr) and originated from high-energy galactic particles and their secondary radiation.

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TABLE I.- EXPERIMENT M191 TLD RESULTS[a]

Cuvette TLD chip sample number	Dose	
	Joule/kg	Rads
Unvented tier		
7121	0.0048 ± 0.0002	0.48 ± 0.02
7122	.0049 ± .0003	.49 ± .03
7123	.0048 ± .0002	.48 ± .02
7127	.0050 ± .0001	.50 ± .01
7128	.0051 ± .0001	.51 ± .01
7133	.0048 ± .0003	.48 ± .03
	Average: .0049 ± .0001	.49 ± .01
Vented tier		
7135	0.0044 ± 0.0005	0.44 ± 0.05
7132	.0049 ± .0002	.49 ± .02
7131	.0047 ± .0001	.47 ± .01
7130	.0049 ± .0004	.49 ± .04
7124	.0047 ± .0002	.47 ± .02
7129	.0048 ± .0002	.48 ± .02
	Average: .0047 ± .0002	.47 ± .02
Flight control tier		
7157	0.0046 ± 0.0001	0.46 ± 0.01
7151	.0047 ± .0002	.47 ± .02
7145	.0048 ± .0005	.48 ± .05
7136	.0048 ± .0004	.48 ± .04
7154	.0047 ± .0002	.47 ± .02
7159	.0046 ± .0003	.46 ± .03
7155	.0046 ± .0003	.46 ± .03
	Average: .0047 ± .0001	.47 ± .01
Average of all samples:	0.0048 ± 0.0002	0.48 ± .02

[a]Differences between tiers and between individual samples are not significant statistically.

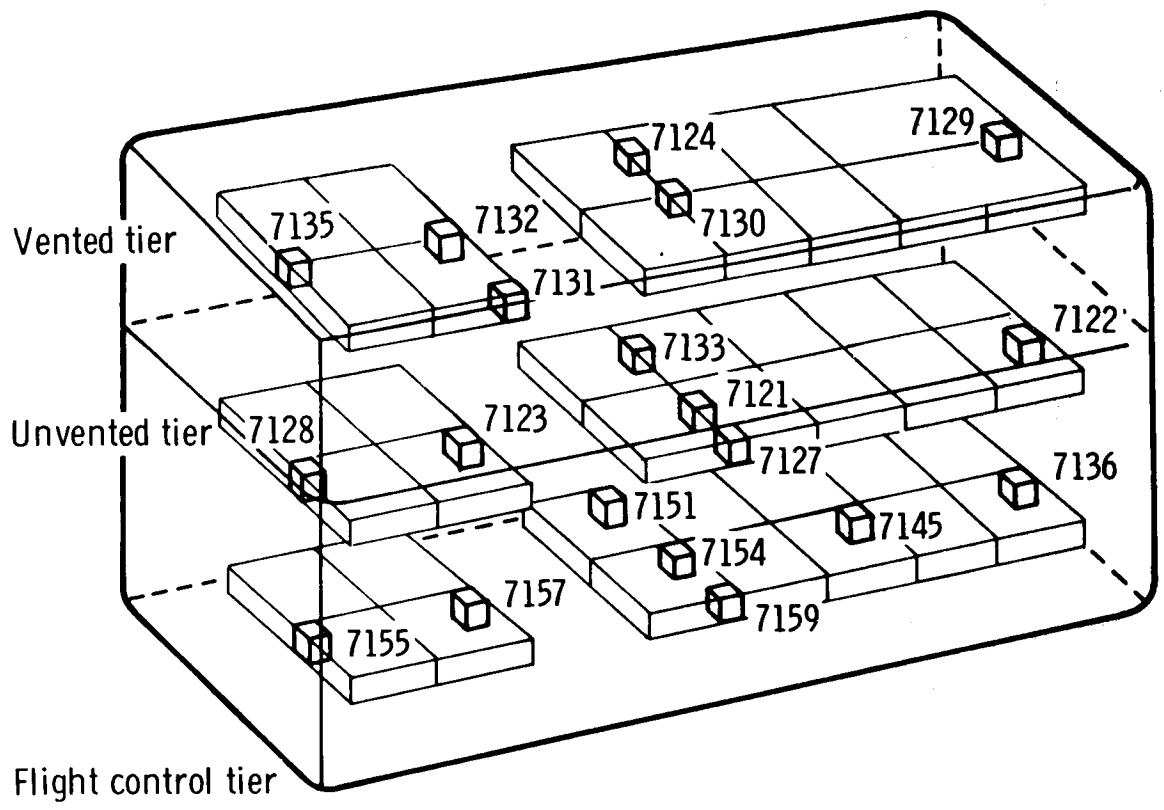

Figure 1.- Arrangement of numbered TLD chip samples within flight hardware.

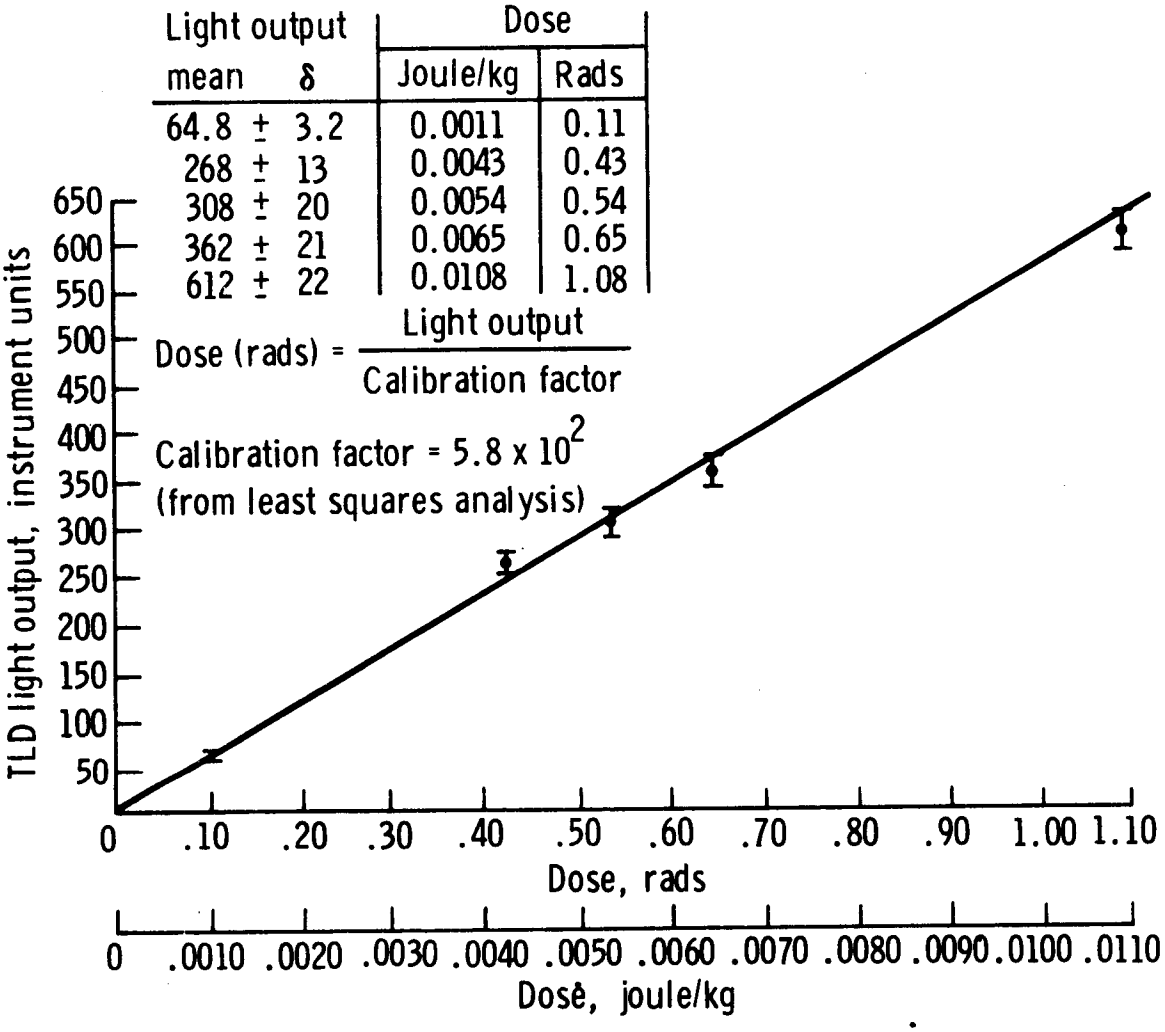

Figure 2.- Experiment M191 TLD calibration.